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ALLERGY

A CHEMICAL CONCEPT OF THE ORIGIN AND DEVELOPMENT OF LIFE A PRELIMINARY PRESENTATION*

By VICTOR C VAUGHAN M D WASHINGTON, D C

THE concept of the origin and development of life which as a result of your kind invitation I am to present to you this evening, has not been evolved from my inner consciousness but has resulted from more than twenty six years of experimentation in my laboratory, modified by the work of others. In the nineties I was seeking a method by which I might obtain large quantities of some low form of life free from contamination. This quest ended in my devising my large bacterial tanks, with which I was able to secure pure bacterial substance by the kilogram, and was able to demonstrate the following fundamental facts:

- 1 Bacterial substance consists of glyconucleoprotein
- 2 It contains no cellulose. Consequently bacteria are not plants
- 3 Bacterial substance shows no differentiation into cytoplasm, nucleus or nucleolus and undergoes no mitosis consequently bacteria are not "cells" as the morphologist would interpret this term
- 4 On cleavage with acid or alkali, bacterial substance yields carbohydrates, aminoacids and purine bases
- 5 It may be split into poisonous and nonpoisonous portions, with evidence that the cleavage follows definite chemical lines
- 6 Dead pathogenic bacterial substance kills animals with the same symptoms and like lesions to those which follow inoculation with the living organism. Therefore the symptoms and lesions of a disease such as typhoid fever

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are not due directly to the growth of these bacilli in the patient's body but result from the cleavage of the bacterial substance by some agency supplied by the body of the host

7 Nonpathogenic bacterial substance furnishes as much poison as does the pathogenic. Therefore, immunity to certain organisms cannot be due to the absence of poison in these organisms but must be explained in some other way

8 Vegetable and animal proteins, such as edestin from hemp seed and casein from milk, contain as much poison as do the pathogenic bacilli

9 All proteins contain a poisonous group. It will be understood that none of these poisons are active when given by mouth and are so only when introduced parenterally. This is because protein cleavage in the alimentary canal is different from that occurring in the blood and tissues

I may add that the above statements in all essentials have been verified by workers in this country, France and Germany. I published them in book form in 1913. I am not going farther into the details of my experimental work but will devote my time to the conclusions which I have drawn. Even with this limitation I can only present a preliminary outline, awaiting opportunity to write in further detail

How can we differentiate between nonliving and living matter? What is the earliest manifestation of the acquisition of life? Certainly matter does not cease to be matter when it becomes endowed with life. An atom of nitrogen in ammonia is still nitrogen when it is incorporated in a more complicated protein molecule

I can say with much confidence that the conversion of nonliving into living matter is accompanied by increased molecular lability. By this I mean that the atoms or electrons within the molecule are energized. Their orbits are enlarged. Within their orbits they move with greater speed. Their chemism is intensified so greatly that they are now able to drag into their orbits atoms and possibly molecules which have hitherto been beyond their grasp. In other words the molecules begin feeding on outside matter

All living things absorb, assimilate and eliminate. This means that metabolism or trading in energy begins. Such is the first evidence of life

Have we any idea of the nature of these primitive living molecules? Yes. They were and are protein molecules. There is no life save in proteins. These are polymers of aminoacids. The aminoacids, at least the simplest of them, have been and are today being formed under proper environmental conditions from inorganic substances

Furthermore, each protein differs from all others in its content, kind or position in the molecule, of aminoacids. Up to the present time less than twenty of these bodies have been found in nature but with this small number, numberless proteins are formed, much as all the words in our language are formed by varied groupings of the twenty-six letters in our alphabet. Simple proteins yield only alpha aminoacids on hydrolysis

In my opinion simple proteins are not living. There must be in the living molecular structure a carbohydrate group thus converting a simple protein

into a glucoprotein I have found two carbohydrates in bacterial substance One of these, which exists in some bacteria to the extent of 10 per cent, I believe to be attached to the nuclein group, while the other is part of the protein and is attached to the nitrogen With this glycoprotein we have a battery and this begins to operate under proper stimuli such as heat, light, electricity or the chemical constituents of something in the medium in which the molecular battery exists In other words the stimulus is some form of energy What causes the aminoacids to synthesize I do not know Emil Fischer has, however, synthesized aminoacids and has obtained a product which closely resembles natural protein

Irritability, or reactivity as Ralph Lillie prefers to call it, has long been known as a universal property of living matter This means that the rate at which energy is received and discharged by the living protein may be altered, increased or decreased, by external stimuli which may be brought into contact with it The stimulus may be in the form of food or fuel which brings to the organism, or the living system as the morphologist calls it, energy in the potential form, which is then discharged in the kinetic form Metabolism is regulated by environment Reaction between the organism and its environment is essential to all living matter Without this, life cannot originate or having originated continue indefinitely One can conceive of a piece of chalk or a lump of carbon existing indefinitely without reacting with its environment, without absorbing or eliminating, but one cannot conceive of a bacterium or a yeast cell retaining life indefinitely under these circumstances I am fond of repeating a statement first employed I believe by Allen "Living matter differs from dead in that the former trades in energy while the latter does not"

Still another attribute of living matter is its ability to reproduce itself As I conceive it, the early forms of life must be particulate, not necessarily as this term is understood by the morphologist but in a chemical sense, meaning that living matter maintains its molecular identity in no matter what form or environment it exists The early forms of life must at the same time be small, microscopic or ultramicroscopic, because the reaction between the organism and its environment can occur only when the reacting bodies are brought into immediate contact This holds true whether we consider the lowest or the highest forms of life, whether we subscribe to the cellular or a chemical theory of the origin of life In man the highest form of life contact is just as intimate, the food material being brought to the cells by the blood and lymph This holds whether the energy be brought to the organism in the potential or kinetic form

There can be no question as to the nature and manner of reproduction in the lowest forms of life since we can see and study it in low forms such as bacteria Reproduction occurs by fission

If we assume that there was an Azoic period in the history of the earth a period in which life even in its simplest forms did not exist, and we must assume this if we will accept the geologist's concept of the origin of the earth, then it follows of necessity that there was a certain time at which life on earth began The evidence today indicates that energy derived from the sun

is the original source of life. The chief difference between inanimate and animate matter is in its energy content. The forces in the sun's rays have energized dead matter into life. Perhaps, as suggested by Mathews, the bulk of this energy is carried by the oxygen atoms within the molecule.

As to what was the form of this first life we can but conjecture, but from the evidence, some of which I have just presented you, I believe that we are safe in assuming that life as such did not exist before the evolution of the protein molecule. Every element which makes up the protein molecule exists also in the inorganic chemical world. There was a time when organic chemical compounds did not yet exist. Henry, referring to the possibility of the artificial production of organic compounds, wrote, "It is not probable that we shall ever attain the power of imitating nature in these operations. For in the functions of a living plant a directing principle appears to be concerned, peculiar to animated bodies, and superior to and differing from the cause which has been termed chemical affinity." And yet only a short time after this Wohler succeeded in synthesizing urea.

Moore and Webster appear to have succeeded in synthesizing formaldehyde from carbon dioxide and moisture under the influence of ultraviolet rays and in the presence of an inorganic colloid. It matters little whether, as has been suggested by vonBaeyer, formaldehyde was the first organic substance produced in nature leading toward the development of life. The point is that it has been shown that organic substances may be synthesized in the laboratory from inorganic substances and that such simple organic structures as aminoacids may be synthesized experimentally into compounds closely resembling natural proteins. It makes no difference whether we can now or ever will be able to reproduce each and every step to the ultimate development of life. Failure in no way invalidates our hypothesis any more than our inability to build a star or planet disproves existing views as to the probable structure of the universe.

At some stage in the evolution of life the cell as we know it today came into existence. It is back to this point that the morphologist traces the origin of living matter and beyond this he does not allow himself to go. The doctrine "*omnis cellula ex cellula*" may perhaps hold after the first cell came into being but the chemist cannot accept the cell as the lowest or the original manifestation of life. Nearly twenty years ago I first stated my belief that life is fundamentally chemical and may, indeed probably does, exist in simpler and less tangible forms than the living cell or even the living bacterium which I do not regard as a true cell for it contains no differentiated cytoplasm and nucleus.

It becomes incumbent upon the chemist who denies the contentions of the morphologist to explain how the cell may evolve from simpler forms of life. This we cannot yet do but the work of DuNouy on the surface equilibria of colloids opens interesting fields for speculation. This author presents evidence that the most probable configuration of equilibrium in a protein colloid solution is in the form of a cell. This would present a minimum of free energy compatible with the total energy. If a microscopic droplet of protein solution is

sprayed into the air the constituent molecules of this droplet will proceed to arrange themselves in thermodynamic equilibrium with relation to each other

In this process the droplet will become coated with a surface layer of protein three hundred or four hundred times more viscous than the interior. If the droplet has a diameter of ten microns, equilibrium may be established within four seconds. If the diameter is but three microns, equilibrium is established in about one second. If equilibrium has been established before the droplet completes its fall, the concentrated surface layer will be strong enough to maintain its shape even if it strikes a dry surface. The presence of carbon dioxide or hydrochloric acid gas or ultraviolet rays suffices to render some of the constituents of the protein layer insoluble thus enabling the droplet to keep its individuality even though it fall into pure water.

Assuming as has been stated before that energy and its transformation is one of the dominant characteristics of life we have evidence in the work of DuNouy that the cell may be but the logical consequence of the tendency of the protein molecule or molecules to establish dynamic equilibrium.

What is the smallest form of living substance known? The smallest living structure known today is that entity which has been described and studied in greatest detail by d'Herelle and to which he has given the name of bacteriophage. This living particulate chemical substance is much smaller than the smallest known cell and bears out my hypothesis first stated nearly twenty years ago. d'Herelle gives to the bacteriophage the generic name protobe or first life. It is without doubt the simplest form of life known today but I regard it as not proved that the first life was not even simpler.

The bacteriophage fulfills all the criteria of life. It can assimilate in a heterologous medium, transforming a heterologous substance into homologous bacteriophage substance, a substance distinctively its own. With this function of assimilation it also possesses the function of adaptation to changing environment. Furthermore it possesses the faculties of reproduction and variability.

The substance is antigenic, has the chemical constitution of protein, possesses as great and prolonged viability as bacterial spores and appears to be an electro negative colloid just as are the majority of the bacterial species. The dimensions of the bacteriophage corpuscle are approximately those of the serum globulin micella its diameter being about twenty millimicrons. The substance appears to be thermolabile its virulence being destroyed at about 75° C.

The protein micella is the colloidal unit. It is the smallest particle of matter in the colloidal state. Possibly as d'Herelle states, it is the unit of living matter and cells are constituted of a union of micellae. The bacteriophage is of about the size of a micella.

I do not regard bacteria as the simplest form of life. Their chemical structure is very complicated. They are essentially nucleins and their chief function is to multiply. Whether the individual consists of a single or many molecules I do not know. Probably their structure is multimolecular but if so the chemism between the molecules must be very strong. I know of no way of distinguishing between intermolecular and intramolecular activity.

Bacteria will live under most diverse conditions. They will grow in a medium which contains organic nitrogen only in the form of ammonia. They continue to live or at least to retain life under wide ranges of temperature. When food is scarce they go into a resting or spore stage. They multiply by fission. In them acquired characters such as increased or decreased virulence are transmitted. They are antigenic and can be shown susceptible to classification in groups by their antigenic reactions.

While the bacterial cell is morphologically simple in structure, it is as complex in chemical composition as are the cells of the animal body. I know of no work done since I reached this conclusion more than twenty years ago which throws any doubt upon it. The conclusion that I would draw is either that bacteria are already relatively high up in the scale of life or else that even the simplest forms of life consist of relatively complex aggregations of protein molecules.

The general constancy and immutability of bacterial types is illustrated in the history of epidemic disease. Generally speaking these diseases run true to type through their recorded history, be this short or long. Tubercle bacilli found in Egyptian mummies present the same characteristics and cause the same type of tissue destruction as do tubercle bacilli in the consumptive of today. The characteristic symptoms and lesions of smallpox observed and described by Indian writers before the Christian era show no essential variation from those which manifest themselves in the unprotected individual of today. Through all the centuries there has been no important mutation in the smallpox virus, nor any marked modification in its behavior when introduced into the human body. The most ancient descriptions of the plague are so plainly indicative of the disease as we know it in the present generation that there can be no mistake of the identity of the virus of this disease in most ancient times with that of the present. The pneumonias of today are marked by the same seasonal variation, characterized by the same modes of onset, by like avenues of progress, and by similar results with those seen and described by Hippocrates. Because bacteria and protozoa are low forms of life it has been assumed that they are especially liable to marked mutations involving alterations in chemical composition and, what is of more importance, so far as pathogenic organisms are concerned, in their effect upon man. In my opinion the assumption that bacteria and protozoa readily undergo mutation is not warranted by any facts which can be gathered in a study of the history of infectious diseases. I am ready to assert that there has been less mutation in the tubercle bacillus or the virus of smallpox since the beginning of recorded time than there has been in man and the other higher animals.

We do not know the nature of the filtrable viruses such as that of smallpox, but it is possible that they are of the nature of protobes such as d'Herelle's bacteriophage. There is some evidence that as time goes on we will be able to establish a more definite connection or association between the protobes and bacteria. In the case of the tubercle bacillus for instance there is evidence that ultrafiltrates of the tubercle bacillus contain what appears to be a living virus and the evidence suggests that this is a small granular form

of the tubercle bacillus As to whether this is a tubercle bacillus micella we can only surmise

Having outlined my concept, a chemical concept, of the early development of life I desire now to present to you my interpretation of the manner in which life became differentiated into its many forms My understanding of this complex phenomenon, as I have said, is not based upon pure philosophic induction but upon experimental observation in my own laboratory Before discussing the origin of species, therefore, I must summarize briefly my conception of the life processes as I have observed them in bacteria

The chief function of life is self perpetuation If this function is to continue active, the living substance must be so situated that it can procure nutritive material from its immediate environment While energy is furnished in the available carbohydrates and fats and while water and certain minerals are requisite, the structural and reproductive requirements of the protein molecule are met only by protein material of which the basis is the amino acid

All living substances are protein The nature of the protein differs for every different type of life This difference is due to variation in the number, nature or intramolecular arrangement of the constituent amino acids There may be other differences which present methods have not as yet enabled us to recognize

If the necessary pabulum were always available as pure amino acids and in the correct proportions for the particular living cell, the matter would be simple As a rule however, the available organic food supply consists of combinations of amino acids in varying degrees of complexity, up to the complete protein molecule

In order that the living substance, let us say a bacterium, may assimilate this food it first becomes necessary to disrupt the heterologous protein molecule into its constituent amino acids so that these may be absorbed and built up into the bacterial structure Bacteria secrete enzymes or ferments for this purpose So do all living cells These ferments will digest certain proteins but not all proteins If a living cell is in contact with a foreign protein against which it does not possess a digestive ferment it will gradually evolve a ferment specific for that protein I believe it to be a fundamental law that a living cell in contact with a foreign protein will evolve an enzyme to destroy that protein Many years ago Duclaux showed that *Penicillium glaucum* grown on starch produces invertase only On lactose it produces lactase in addition On milk it elaborates a proteolytic enzyme The ability of living cells to produce specific enzymes to meet the necessity for disrupting the substrate with which they come in contact is essential to existence both under normal and abnormal conditions It enables the cells to feed upon assimilable substances and to destroy injurious ones The ease with which living cells may function in these directions is dependent upon many and varied factors such as temperature physical and chemical conditions, the activity of the cell which is seeking to feed or protect itself and the constitution of the body upon which it acts Here lie many problems awaiting future investigation

Much remains to be learned of the nature of ferments or enzymes They are particles of matter, some of them wholly simple like spongy platinum,

others highly complex like the yeast ferment or pepsin or trypsin. Enzymes are inanimate storehouses of energy which may be brought into action under proper environment or on coming under the influence of certain physical or chemical stimuli. They may be compared roughly to storage batteries. Ferments may be protein, but are not animate.

In the same way that the bacterium will elaborate a digestive enzyme, so also will the body cell do this when it comes in contact with a foreign protein. The typhoid bacillus on entrance into the body grows luxuriantly during the incubation period, for in the blood it finds an abundance of available food material in the same simple form that is available for the body cells. Living typhoid bacilli have been found in the blood of man during the incubation period, before any symptoms of the disease have become manifest. The germ is converting body proteins or at least the aminoacids of the blood into typhoid bacillus protein. The reaction is synthetic and there are no symptoms.

But the body cells have been stimulated by the presence of a foreign protein and in about ten days they have elaborated a ferment or enzyme which will break down this foreign protein. As soon as this defense reaction becomes well developed, disease becomes manifest. Now, typhoid bacilli are being destroyed, the process is analytic, the protein poison is being liberated.

During the incubation period the process is constructive. After the body cells have learned to elaborate a ferment which will destroy the typhoid bacillus the process in turn becomes destructive and in this destruction the protein poison appears to be liberated. This poison I have found to be present in every protein which I have so far examined. It exists not alone in pathogenic bacteria but also in nonpathogenic and even in such otherwise innocuous proteins as egg white and the proteins of the cereal grains. Indeed edestin from hemp seed and casein from milk, and egg white furnished me the largest and most satisfactory amounts of protein poison. While I have been unable to obtain this substance in anything approaching a pure state, it appears to contain many aminoacids and apparently should be classed as a polypeptid. It is only poisonous when administered parenterally, for alimentary digestion apparently further breaks it up into the simple aminoacids. In the case of typhoid fever which I have used as an example the symptoms result from the liberation of the protein poison. The severity of the disease depends upon the amount and rapidity of liberation of the poison. The very small doses of the poison which will produce serious symptoms experimentally indicate its possession of a high degree of energy. In my opinion it kills by tearing off from certain body cells secondary and functioning chemical groups.

The perpetuation of life depends upon the ability of living substance to convert heterologous proteins into homologous proteins. This holds equally for the higher forms of life, for if in the case under consideration the human body is unable to convert typhoid protein into human protein the result will be disastrous. True, this conversion is of itself not without danger.

The ability of a bacterium to produce disease after it has entered an animal depends mainly upon two factors. First, it must be able to establish for itself a parasitic existence in its host. It must be able to sustain itself and multiply its kind on the pabulum within its reach. Second, there must

be no destructive enzyme already existing in the body tissues, against this particular bacterium

Disease depends in great part upon how abundantly a given microorganism may multiply in the tissues before the body cells have completed the elaboration of a destructive enzyme. Where one is already in existence the bacterium is destroyed at once and only an infinitesimal amount of poison is liberated. No symptoms result. The seriousness of the symptoms depends upon the amount of poison liberated and the rapidity of its liberation. Of course, there are other factors in certain diseases such as location within the body, the secretion of a toxin by the bacterium and the like.

Let us now take up a consideration of those factors which may have had a bearing in the origin of species. At this point I am not so interested in the inheritance of identical characteristics as I am in the inheritance of altered or acquired characteristics, for it is by virtue of the latter that new species develop. I find no great difficulty in understanding that living substance might readily reproduce itself in its entirety but I am highly interested in the intimation that it can produce another living substance different from any that has been known heretofore.

I have said that the characteristics of bacteria have remained remarkably constant throughout the history of disease. There is an exception, one which I believe to be of fundamental importance in the development of species. I have said that the tubercle bacillus as it occurs in man appears to have undergone no remarkable change through recorded history. This is quite true but at the same time there is a tubercle bacillus which infects fowls which is not quite the same germ and yet another which infects cattle. Dr. Calmette has at the Pasteur Institute in Paris a strain of tubercle bacillus which he has cultivated artificially for over thirteen years and which appears to have lost entirely its ability to infect man and the lower animals.

The constancy of bacterial types and indeed of all living substances depends upon a relatively unchanging environment. In the lower forms of life environment has a very definite influence upon the characteristics of life. Furthermore, alterations in the structure of the protein molecule resultant on environmental changes may be and are inherited. A microorganism living in a milieu in which the pabulum is readily assimilated and transformed into homologous proteins, will thrive. If, on the other hand, the environment is one in which the available food material is of widely different constitution from that of the living substance continued existence will depend upon the ability of the microorganism to elaborate a ferment capable of disintegrating the foreign protein or protein like substance into its constituent aminoacids so that they may be available for assimilation. If some of these aminoacids are deficient in quantity for the particular living substance, continued existence will now depend upon the ability of the living structure to adapt itself to this deficiency. If such an adaptation is made, there will result a change in the make up of the living protein molecule.

While I am emphasizing chemical factors I am not unmindful that physical and other factors also play a part. I can readily understand why many species of animals and of plants have disappeared. No species can continue

when it ceases to receive and utilize energy from its environment. A change of a few degrees in the annual average temperature might change markedly the flora and fauna of the area in which it occurs. Climatic factors are more readily recognized in the higher forms of life, but I shall continue to limit myself to the effect of changes in the chemical environment on the lower forms of life.

The presence and availability of new or different aminoacids or similar protein radicals will ultimately determine an alteration in the constitution of the living protein molecule. If this alteration in environment is permanent the altered constitution of the living molecule will likewise become permanent and will remain so as long as the environment is relatively the same. The development of new species in the lowest forms of life depends upon physicochemical alterations in the environment. The persistence of new species so formed is dependent upon the permanency of the environmental changes.

A streptococcus highly pathogenic for the horse will on repeated passage through a laboratory animal such as the mouse or rabbit or guinea pig, gradually lose its high virulence for the horse while acquiring an increased invasive power against that animal through which it is being passed. That there is an actual change in the chemical constitution of this streptococcus is indicated by the fact that after several passages its antigenic power as a horse streptococcus which was originally of high titer becomes completely lost.

Not only this but it has been found that cultivation of a streptococcus in an artificial medium containing the blood of some laboratory animal increases the virulence of the streptococcus against this particular animal. Furthermore the antigenic characteristics of this streptococcus are altered after repeated growth on these special laboratory media. Thus we may speak of a horse streptococcus, a mouse or rabbit or guinea pig streptococcus all derived from the same ancestor, each of them still a streptococcus, but definitely altered in chemical nature by the immediate nutritive environment.

d'Herelle believes that there is but one bacteriophage but that this like the streptococcus just described is capable of adaptation to growth in a wide variety of bacterial hosts. The Shiga bacteriophage and the Staphylo-phage differ from each other in their predilection one for the dysentery bacillus and the other for the staphylococcus. These are their foods of choice and they find it difficult to grow on other bacteria. However, adaptation may be accomplished and it is possible to change the Shiga-phage into the Staphylo-phage and vice versa. There is an almost limitless possible number of bacteriophages dependent upon the degree of invasiveness for different bacteria but the evidence presented by d'Herelle would indicate that this is a matter of adaptation to the environment on the part of a single original bacteriophage.

This adaptation is so complete that it involves an alteration in the chemical structure of the bacteriophage, which can be recognized in changes in its antigenic properties. Shiga-phage actually becomes chemically different from the Staphylococcus bacteriophage. Alterations in the environment have produced a new species which will maintain its identity as long as the en-

environment remains essentially unchanged. Further environmental changes will produce yet other alterations in the structure of the living molecule, not necessarily a reversion to the original structure but perhaps with the development of an entirely new and more complex structure to suit the requirements of the altered nutritive environment.

Many of the higher forms of life contain two or more proteins, no one of which can be said to be more specific than the other for that particular living substance. Such a simple plant as wheat for example contains gliadin, globulin, glutenin, proteose, and leucosin, five proteins in all.

Now wheat glutenin appears to be similar in its chemical constitution with the glutenin found in barley and in rye.

I would interpret this as highly suggestive evidence that wheat, barley and rye evolved from the same primordial ancestor. Environmental changes, possibly variations in the nutritional resources have been responsible for the differentiation of these three grains. The farmer of today knows well the importance of environment. With the same seed, the same heredity, he does not anticipate an equally good or abundant crop in every field in different years. The fertility of the soil, the amount of sun and rain and many other environmental factors play a most important part. If some of these factors are disadvantageous to the continued existence of a grain, this grain must either adapt itself to changed conditions which it will do with alterations in its own structure and appearance or it must eventually die out.

In forms of life such as those which we have just been discussing, in which two or more different proteins exist together, we must conceive as possible that species differentiation does not necessarily entail complete change in any or all of the constituent proteins but that in these higher forms new proteins may be added, possibly by differentiation of the original with the result that the new protein and the original both exist in the same living substance.

Where a protein has at last been evolved which best fits the functional needs and where its environment remains little changed, its chemical constitution will remain remarkably constant. It is said that the proteins of the lens of the eye are different from the other proteins of the body but are identical in the lenses of a wide variety of animals. Here there is little environmental change for the environment is not the outside world but the blood and lymph.

I have mentioned changes in antigenic properties as indicating alterations in the make up of the protein molecule. The question might be raised as to whether such changes do necessarily indicate alterations in the constitution of the molecule. The term antigen is employed by immunologists to designate those substances which when introduced into the animal body parenterally lead to the elaboration within the treated animal of a substance which antagonizes or tends to neutralize its own action. Up to the present the weight of evidence is all that antigens are proteins. Moreover it appears that each protein leads to the elaboration of a specific antibody. Thus an animal treated with the venom of a certain species of snake produces an antibody to this venom and not to the venoms of other species of snakes. The

toxin of the diphtheria bacillus produces a diphtheria antitoxin and this has no antagonistic action on other toxins. Each antigen acts specifically and the nature of the antibody formed is strictly specific. The body cells appear to elaborate these antibodies and they do it for self-protection. Some of the antibodies neutralize their specific antigen by combining with them and thus rendering them inert. This seems to be true of the antitoxins of diphtheria and tetanus, also for vegetable toxins such as those of abrin and ricin and the venoms of snakes. In other cases the antibody renders its antigen inert by disrupting it into its harmless constituents. Could there be better or more conclusive evidence of the ability of the body cells to adapt themselves to their environment and to protect themselves against threatened destruction? Living cells are capable of being trained or educated. In other words their behavior may be modified by changed environment.

It has been shown that the specificity of antigens is dependent upon their chemical composition. For instance there are in milk four chemically distinct proteins and each is capable of causing the body cells to elaborate its own specific antibody. In egg protein there are three chemically distinct proteins some of which are common to the eggs of different species of birds while others are found in a single or in a limited number of species.

It seems to be true that the specificity of an antigen is determined by the location of some aromatic radicals within the structure of the protein molecule. When proteins are hydrolyzed they lose their antigenic properties. My students and I showed many years ago that gelatin which is a hydrolyzed protein and devoid of certain aromatic radicals such as tyrosin and tryptophan, and which contains only a trace of phenylalanin is not an antigen. Like results have been obtained by subjecting true proteins to the cleavage action of digestive ferments such as trypsin. Likewise the protamins, which are complexes of diaminoacids, and wanting in the aminoacids, are not antigenic. Free aminoacids are not antigenic. All antigens are colloids, all apparently are proteins.

Now, it has been found that certain chemicals, as formaldehyde, nitrous acid and iodine may be introduced into the protein molecule without destruction to its antigenic properties, but the antibodies elaborated are specific to the altered proteins and not to the original substances. Some years ago Obermeyer and Pick found that the serum of rabbits treated with proteins which had been radically changed by being iodized or nitrified did not precipitate the native protein but did act upon the altered protein with which the animal had been treated.

All life is protein and the development of new species is due to molecular rearrangements in the structure of the protein molecule. Something is added, or subtracted, or chemical groups within the molecule are rearranged. The recently discovered facts demonstrated by the precipitin and sensitization tests make this certain. By these, proteins may be positively identified when mixed or when unmixed with other proteins. Group relationship may be shown by these methods and up to the present time in no other way. Especially is this true when the results of these tests are measured quantitatively. The proteins of the hen's egg sensitize guinea pigs to themselves and to a

lesser degree to the proteins of the eggs of other birds. The proteins of man's blood sensitize animals to themselves and less perfectly to those of the blood of the anthropoid apes. Wheat, rice and barley all come from a common plant and under different environments have developed into three species. In this way varieties and species come into existence.

From the lowest to the highest forms of life environment plays a part of greater or less significance in the development of species. These environmental factors may be chemical or they may be physical. I have presented to you my concept based upon the simpler forms of life for here that very simplicity facilitates more accurate study and interpretation.

In calling to your attention the primary importance of environment in the development of life and the differentiation of species it is in no way my desire to intimate that I am not in accord with the prevailing doctrines of heredity. The discussion is along quite different lines for in the latter our interest is in phenomena in which gross alterations are conspicuously absent while in the former it is the alterations which are of chief importance and interest. The genes about which students of heredity are saying much I can accept, if I am permitted to regard these genes as atomic groups, some right handed, some left handed in the specific protein which reproduces itself. Apparently this has been done by Jennings and by Ralph Lillie.

But I find no difficulty in recognizing the action of chemical environment even in the highest forms of life. Morphologists stress the stability of germ plasma but some of them do admit that certain poisons such as alcohol, lead, mercury, and syphilis may deleteriously affect the reproductive cells. In my opinion even more striking examples might be given. A boy and a girl born of healthy parents and raised to maturity under normal conditions may migrate into a goiterous district and after acquiring goiters may marry. Their children may be cretins. In this case it is the *absence* according to the now accepted belief, of iodine in the food and drink which leads to this deterioration. Please understand that it is only the absence of one chemical element which causes this disaster.

I am in favor of eugenics but I cannot forget that environment as well as heredity must be taken into consideration.

The claim that the reproductive cells are not influenced by the somatic cells is one which I believe to be unwarranted. In seedling it is well to select sound grain but the harvest will not be determined wholly by this but will depend to some extent on the fertility of the soil.

What is the optimum relationship between the chemical environment, particularly the food supply and the living structure? Without considering other factors that undoubtedly play a part I would say that the more closely the heterologous protein resembles in its make up the homologous living protein the more nearly identical its content and proportion of the different amino acids and associated radicals the more constant will be the composition of the living molecule and the higher will be the degree of perfection which it will attain while remaining essentially unaltered chemically. I recognize that there are other essential requirements such as vitamins and the

like but the basis of life is protein and in this thesis I have limited myself almost entirely to the consideration of the chemistry of the living protein molecule

Some living forms such as bacteria feed upon other living forms. They can do so because they can convert and assimilate without difficulty the protein molecules of the host. I consider it possible that the more nearly the proteins of the host resemble chemically those of the invader the greater will be the pathogenicity of the latter. I suggest that some investigator study the antigenic reactions, the protein relationships between parasite and host. In the case of bacteria, feeding upon man or animal, the objection might be raised that they derive their sustenance, not from the living molecules of the animal, but from the simpler protein food radicles and cleavage products, present in the circulating blood and lymph. But this objection cannot hold in the case of test tube experiments in which the available pabulum consists of the tissues of the host.

One might infer that I believe that a cannibalistic existence would be the ideal form of life. But curiously enough even in such low forms of life as bacteria and bacteriophages, cannibalism appears not to exist. Protein molecules endowed with attributes of life, while apparently bent upon the destruction of other forms of life, particularly simpler forms, appear incapable of destroying living substances of identical or nearly identical chemical constitution. This is readily understandable. Where there are two such living substances in apposition, their chemisms would be identical, their spheres of influence the same, then tropisms would balance one another and the result would be no chemical reaction. A solution of ammonium chloride mixed with another solution of ammonium chloride remains ammonium chloride.

Late in the eighteenth century Lavoisier, scientist, patriot, martyr, showed that the process of respiration in man is comparable to the burning of a candle. About 100 years ago, Wohler made urea synthetically. A few years later Dunghlsson and Emett, in their scantily supplied laboratory at the University of Virginia announced that the free acid in the gastric juice of man is hydrochloric acid. Dumas in France, Liebig in Germany, and others continued to develop physiologic chemistry. About the middle of the last century leading universities in this country provided chairs in this subject. For many years Chittenden at Yale was the standard bearer and on his retirement his good work was continued and amplified by Mendel. I gave my first lecture in physiologic chemistry in the University of Michigan in January, 1876. Splendid work in this subject has been done by Van Slyke, Lusk, Folin and others. Ehrlich and Hatta, after more than 600 attempts built up arsphenamine synthetically and this with his cogeners has done much to mitigate the plagues of syphilis and allied diseases.

Starling and others have discovered hormones and the brilliant results obtained by Banting in his discovery of insulin are well known. Abel not only discovered epinephrine, but determined its structural formula and it is now made synthetically. The same talented investigator appears to be on the high road to similar results in the study of insulin.

All these, however, are inanimate substances and up to the present time no chemist has awakened dead matter into life. It may be that this will never be done, although I do not consider it beyond the bounds of possibility. Whatever may be individual opinion on this subject, past, present and even future, failures should not prevent us from interrogating nature and learning so far as possible how she in her great laboratory with boundless facilities and with countless ages in which to operate has accomplished this great result. Without predictions as to what degree of knowledge future researches will reveal I have ventured to present my views on this subject. Should they even in part be confirmed the morphologist must radically change his teachings as to the relative importance of heredity and environment. I hold that the lowest forms of life have come into existence through chemical agencies and that environment has been a stronger factor in the evolution of life and in the development of the varieties and species than is believed by the biologist of today.

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SENILE PRURITIS DUE TO HYPERSENSITIVENESS*

BY JAMES WYNN, M D, INDIANAPOLIS, IND

REPORT OF CASES

CASE 1—In May, 1923 Mrs W, a widow aged 69, consulted me for the relief of obstinate generalized itching of eighteen months' duration. The trouble, beginning insidiously as a mild tingling of the knuckles and fingers, had become in the course of a few weeks a severe generalized pruritis, associated with recurrent attacks of nausea, vomiting, and right upper quadrant abdominal pain. Various diagnoses such as senile pruritis, "biliousness," and "gall" poisoning had been made. Cholecystectomy had been advised and refused. During the month prior to consulting me she had been taking heroic doses of alkalies and spending at least three hours of every night in a soda bath—the only procedure short of an opiate that enabled her to sleep even fitfully.

Possessing keen mind and good memory, Mrs W was able to give a quite complete family and personal history, no features of which suggested the allergic diathesis in her mother and father, brother and sisters, or herself. Her parents, still living and surprisingly free from either physical or mental evidence of senility, were interviewed and gave a detailed history revealing no suggestion of allergic symptoms in their parents. Mrs W had two grown children and six grandchildren. Her two sisters also had grown children, one of them, a grandchild. In none of these individuals were there any clinical evidences of the allergic constitution.

The record of Mrs W's personal ailments included the usual childhood diseases and little else except an attack of what had been called colitis (from June to October, 1922. This terminated about eight months before the onset of the itching). The symptoms had been frequent, pultaceous stools and crampy abdominal pain. At this time routine examination revealed high blood pressure and arterial disease, and a purin free diet was instituted. Since giving up meat, she had lived largely on green vegetables, milk, and bread—eating never less than five or six slices of bread a day.

Physical examination revealed moderate obesity, hypertension (190/100), definite peripheral sclerosis, and thickening of the retinal vessels. Except for slight puffiness of the ear lobes, the skin surface was entirely free from such abnormalities as jaundice, eruptions, wheals, thickening, or induration. Scratching with a blunt object led only to momentary redness, no wheals. Blood examination revealed a definite eosinophilia but no elevation in the urea nitrogen, uric acid, or bilirubin content. Skin tests (Walker's method) with all the foods in her diet and various epidermal and pollen extracts were all negative with the single exception of wheat globulin, which produced a wheal 2.5 cm in diameter which persisted two hours, itching violently.

All bread, crackers, and wheat containing foods were at once excluded from Mrs W's diet, and within twelve hours there was complete cessation of itching. She remained free from any cutaneous or gastrointestinal symptoms until seven months later when she one day ate two slices of white bread at noon lunch. By three o'clock she was having crampy abdominal pain which persisted intermittently throughout that afternoon and night. Itching of the hands and face kept her awake most of the night, gradually disappearing toward noon of the following day. There was no urticaria and aside from slight puffiness of the ear lobes no angioneurotic edema.

*From the Department of Medicine Indiana University Medical School

Read by title before the American Association for the Study of Allergy, Washington

D C April 16 1927

On two subsequent occasions during the following year (1924) Mrs W attempted to eat bread, but each time with generalized reaction similar to the episode just described. Thereafter, carefully excluding all forms of wheat from her diet, she had no further itching, and remained otherwise symptom free until her death following a cerebral hemorrhage in January, 1925.

CASE 2—The case of the Widow B, aged 70, is so similar in general features to the one just reviewed that only the points of difference will be summarized. Mrs B's parents, grandparents, one grown son (the only child), and two grandsons gave no evidence of the allergic diathesis according to detailed and in every case carefully confirmed histories. Mrs B's itching was less severe than that of Mrs W but had persisted unintermittently for two years (June, 1921 to June, 1923). Five months before its onset (January, 1921) she had had a nine weeks' siege with "intestinal influenza" (mild diarrhea, soft stools, crampy abdominal pain). She had always been an excessive cake and bread eater.

Complete physical and laboratory examination of Mrs B revealed nothing but obesity, the evidences of old age, some puffiness of the ear lobes and a positive wheat globulin skin test. (There was no eosinophilia as in the first case.)

Wheat globulin free diet proved just as effective as with the first patient. There were no further symptoms except one day of itching following the ingestion of bread at a family reunion about six weeks before her terminal illness with bronchopneumonia (November, 1924).

COMMENT

These case histories are cited to illustrate three clinical facts, a more general recognition of which should be helpful to dermatologist and internist as well as to the specialist in geriatrics. These facts may be briefly stated: (a) generalized pruritus, due to specific hypersensitiveness, may first manifest itself quite late in life, (b) except for a scarcely perceptible puffiness of the ear lobes, it may be entirely unassociated with any cutaneous physical signs, (c) it may occasionally occur in individuals with no personal history suggesting latent atopic hypersensitiveness and no such family history in at least two antecedent and subsequent generations.

In the cases here reported dietetic experiment as well as skin tests clearly indicate the etiologic role of wheat globulin hypersensitiveness. (The histories and blood chemical analyses pretty definitely exclude the influence of psychic factors¹, purin metabolism disturbances². The observation of urticaria (and less commonly uncomplicated pruritus) in patients of the atopic hypersensitive type is a matter of every day experience to any one working extensively in this field. The history in such cases, however, usually reveals recurrent attacks dating back if not to childhood, at least to early adult life, and there are other clinical and familial evidences of the allergic or atopic hypersensitive diathesis. The two cases here reported are conspicuously atypical in these respects. Lurthlen⁴ mentions hypersensitiveness to foods as a cause of senile pruritus, but gives no case histories which might enable one to judge from the hereditary features and the occurrence of related allergic syndromes as to the existence of true atopic hypersensitiveness.

Rackemann,⁵ reviewing a series of cases where occupational exposure was followed by the development of hypersensitiveness (a chemist to azofuchsins, a baker to wheat, etc.) suggests that a constitutional "tendency" must exist to account for the relative rarity of sensitization in the various occupational groups. He concedes the possibility of an hereditary influence

in determining this tendency, but in view of the inconstancy of suggestive family histories and in his orris root and horse dander cases, he does not attach such significance to heredity as is accorded it by Cooke⁶ and others. Despite Coca's⁸ evidence that the atopic reaginogenic function is subject strictly to the familial influence demonstrated by Cooke and VanderVeer,⁶ and Spain,⁷ various European observers,^{9, 10, 11} have even further minimized the importance of heredity as a factor in hypersensitiveness. Von Leeuwen¹¹ states that while hypersensitiveness may unquestionably manifest itself in individuals with a certain inherited tendency, it may also develop in those without trace to predisposition, provided exposure is long continued under conditions facilitating the entrance of the "alleigen" through the skin or mucosa. Briefly stated, the "facilitating conditions" are (a) lowered resistance (e g, as from eczema, bronchitis, etc), or (b) a peculiarly irritant allergen (e g, the histamine-like substance in pollen). Von Leeuwen cites Ancona's peasants¹² as classically illustrating both factors at work: these millers handled deteriorated grain (the irritant factor) containing among other parasites *Pediculoides ventricosus*, which set up a common skin disease probably lowering local resistance (the penetrability factor). After a time all the continually exposed individuals developed asthma and urticaria, the factor of predisposition dropping entirely out, as Von Leeuwen emphasizes.

At least some factors in the cases here reported suggest the existence of Von Leeuwen's facilitating conditions for sensitization. For a considerable period these old ladies had ingested relatively large amounts of wheat-containing foods. For a varying period of months while on this diet each had had an intestinal disturbance characterized by frequent loose stools, excessive flatulence, and crampy abdominal pain. The duration of this syndrome, it seems to me, may be taken to predicate definite mucosal changes, abnormal intestinal flora may conceivably represent Von Leeuwen's second or "irritant" factor.

Dubois, Schloss, Anderson,¹³ and Walzer,¹⁴ however, have recently demonstrated that even the normal intestinal wall is astonishingly permeable for foreign protein, that nonsensitive human subjects may have a considerable power of producing anaphylactic antibodies whereas the blood of naturally sensitive (atopic) human subjects may lack such antibodies entirely. The question naturally arises—may not the cases here reported represent a condition closely resembling genuine anaphylaxis and quite distinct and fundamentally different from the atopic hypersensitiveness so clearly defined by the able researches of Coca and his associates? May not the cases of Ancona (quoted as so significant by the European observers) belong in the same near-anaphylactic, nonatopic category—genuine instances of acquired rather than natural hypersensitiveness? Such an hypothesis will explain, at least in part, some of the existing controversy over the relationship of atopy to other forms of hypersensitiveness.

Unfortunately both of the patients here considered died before Coca and Grove¹⁵ had announced their conception of the atopic reagin, before Walzer and Kramer¹⁶ had reported their ingenious indirect method of testing for conditions of atopic hypersensitiveness. Further work in the light of these

observations was hence impossible. In the future, however, such work on patients of this type should do much to clear up the present confusion of opinion on these fundamental issues.

SUMMARY

1 The case histories reported indicate (a) general pruritis due to specific hypersensitiveness may first manifest itself quite late in life, (b) except for scarcely perceptible puffiness of the ear lobes it may be entirely unassociated with any cutaneous physical signs (c) it may occasionally occur in individuals with no personal history suggesting latent atopic hypersensitiveness and no such family history in at least two antecedent and subsequent generations.

2 Certain clinical features are cited which suggest that these patients are nonatopic and have developed an artificial hypersensitiveness more closely related to anaphylaxis than atopy.

3 The question is raised as to whether Doerr and Von Leeuwen, basing their arguments for the fundamental identity of atopy and anaphylaxis on Auconna's asthma epidemica may not be dealing with examples of a general process of artificial sensitization similar to that which is exemplified in these cases of pruritis.

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MENTAL AND NEUROLOGIC REACTIONS OF THE ASTHMA PATIENT*

BY W W DUKE, M D, KANSAS CITY, MO

PATIENTS with hay fever, asthma, or urticaria are inclined to be different from the normal so far as their neurologic reactions are concerned. This has been noticed and mentioned repeatedly both in the earlier literature and in recent literature. The earlier writers stated, in substance, that hay fever is a disease ingrafted upon patients having a neuropathic background. Some of the more recent writers on psychoanalysis include hay fever, asthma, and urticaria as symptoms characteristic of psychoneurotic disorders.

This paper is based upon an observation of mental and neurologic symptoms occurring in individuals under treatment for hay fever, asthma, urticaria, or eczema. I prefer presenting the paper as one based upon impressions rather than as one based upon scientific study because of the fact that my knowledge of neurology and psychiatry is so meager that I would not venture to present anything in this field as amounting to more than an impression.

It has long been known and has been proved by Cooke and Vandever, Coca and his associates that the symptoms and reactions which they term atopy depend upon an inherited peculiarity of the individual. Their statements, I think, are accepted almost universally and harmonize with the experience of practically every careful observer. Symptoms are the result of an abnormal, or at least unusual, reaction on the part of the patient to certain agents to which he is sensitive. The pathogenesis of the reaction, if I understand Coca correctly, is twofold: first, a combination between atopen (the agent to which the patient is sensitive), and reagin (a specific substance in the tissues of the atopic patient with which the atopen combines), and second, the quick unusual reaction on the part of the sensitive patient caused by the combination between atopen and reagin.

I have observed and described in detail two groups of patients having exactly the same type of reaction and the same heredity who differ from the above in the important detail that their symptoms are not caused primarily by sensitiveness to a material agent, such as pollen or fish glue but instead to a physical agent, such as light, heat, cold, or mechanical irritation. I described this under the noncommittal term "physical allergy."

Acquaintance with patients of either type makes one realize that the unusual reactions are not confined to abnormal reactivity on the part of nonstriated muscles, secretory organs, and other tissues ordinarily dealt with in the literature but that they seem, in addition, to occur rather frequently in one or more portions of the central nervous system and to cause the patient to react in abnormal ways to certain specific material impressions and to

*Read before the American Association for the Study of Allergy, Washington, D. C., May 23, 1927.

sensations Reactions of the latter type are inclined to involve certain classes of nerve cells and to vary in different individuals They are not a prominent peculiarity in all patients but can be observed, if sought for, very frequently in hay fever, asthma, and hive cases, including both the atopic types as defined by Coea, and in both physical types as well In substance, these reactions cause mental and neurologic reactions in response to certain impressions or sensations which appear as an exaggeration of that which would appear in a normal individual

It is not an exaggeration to say that when I converse with parents of an asthmatic child about the child's illness I can usually predict correctly whether the illness in the child is inherited from the mother or father, or from both sides of the family This would seem a broad and useless statement if I were unable to state exactly the characteristic upon which such an opinion can be based As a rule, individuals of atopic strain show an exaggerated mental response to matters which concern them deeply The illness of the child very naturally concerns parents deeply If one discusses a child's illness with a normal parent the response is about the same as it appears in the parents of nonasthmatic children A similar conversation, however, with a parent from whom atopy is inherited brings forth usually an exaggerated response, frequently so marked as to make discussion with that parent rather disagreeable In cases where this characteristic is extreme, it may interfere seriously with the welfare and the successful treatment of the child This can be well illustrated by the following case

A girl about six years old was brought to me with a history of asthma of several years' standing She had been referred to me by a physician, the mother's brother in law Because of limited means there had been much discussion between the doctor and the parents as to the advisability of taking on additional expense which would further burden the family The child was finally brought in by the mother When the examination was half completed, the mother suddenly decided that the child's welfare demanded that she be taken immediately to a desert region and there to remain at least two years She stated to the father and to her brother in law that I had done all I could whereupon, against the advice of the husband, brother in law, friends, and myself, she disappeared with the child on a day's notice to seek comfort elsewhere Such a reaction could be neither beneficial nor economical

Hay fever and asthma patients sometimes react in a similar way in matters which concern their own illness or financial status and frequently before an examination is completed or before treatment is finished they decide suddenly to seek some phantom remedy which actually promises almost nothing

The above peculiarity frequently makes the asthma patient a difficult one to handle, in fact, the greatest struggle which I have in obtaining the cooperation of patients is in those subject to hay fever asthma or hives in whom this disposition is prominent Patients with tuberculosis cancer, or other serious diseases will usually go through almost any ordeal recommended by the physician if the necessity for this ordeal is placed before them logically This, unfortunately, is not true of a certain proportion of hay fever, asthma and hive cases

A mental reaction of a different type, namely, a change in personality, occurs rather frequently in patients with asthma during their attacks. This applies to perennial cases rather than to seasonal cases. It can hardly be attributed to discomfort caused by the symptom asthma, because of the fact that it frequently becomes manifest when the symptom asthma, is not marked. It appears to be a change in personality which makes the patient irritable and makes him give mental responses totally different from those which occur in normal individuals or in the same individual during good health. A most striking example of this was a boy about eight years old who had been nicknamed Sunny by his parents and friends because of his sunny disposition. When I examined the child during an attack of asthma, his disposition was anything but sunny, in fact, he was hopelessly unmanageable, fighting parents, friends, and doctor. I was astonished upon seeing him later during a well period to find an extremely pleasant, likable little chap, obedient and willing to cooperate in any way with all of us. I have learned that it is a mistake to judge the nature of a patient during an attack of perennial asthma. A patient who may appear despicable during an attack may be very likable when well. Occasionally patients appear actually hysterical during their attacks. One patient, a man of about sixty, with slight asthma, had a peculiarity in respiration which appeared very remarkable and entirely voluntary. I had to leave the office soon after seeing him. About one-half hour later I received a telephone message from my assistant stating that the patient appeared to be dead. When I returned, the patient appeared about as he had when I left. It was stated, however, that soon after my departure he was suddenly seized with difficult breathing, became comatose to the extent that he could not be aroused, and soon was deeply cyanotic, pulseless, and appeared lifeless, in fact, my assistant, a doctor of considerable experience, believed that he was dead. The same type of reaction with similar recovery occurred later when I left the office. The following day the patient sought relief in a different climate.

An egg and feather case who had travelled a considerable distance to consult me, regained almost perfect health through avoidance of egg and feathers. She came to the office once during a slight attack and upon being sent into an examination room, promptly placed her head between two pillows and remained there until she was asthmatic to such an extent that she required the attention of a nurse and a physician throughout the night. When asked why she did such a ridiculous thing as to smother herself in pillows when she knew she was sensitive to the feathers, she stated that when she entered the room she scarcely knew what she was doing.

In a previous paper I described at some length symptoms similar to those caused by pollen reactions except that they were caused by heat and in many instances by change from undue cold to an atmosphere which is relatively warm. The same reaction could be caused by the heat generated by either mental or physical activity and was often more pronounced if the patient had previously been at rest or was cold. I have observed a number of patients with identical reactions caused by mental activity and in many cases the reaction is augmented by a previous state of mental depression. A pronounced

case of this sort was that of a large, jolly woman with urticaria who would invariably get well upon coming to Kansas City and who would have an immediate recurrence upon returning home. To make a long story short, she had a husband with Parkinson's disease who required her constant care. The situation at home was necessarily depressing to a woman of her jovial disposition and while there outbursts of temper caused by the stupidity of her husband or attacks of laughter brought out by interesting company would cause an outburst of urticaria and angioneurotic edema which would make her life miserable for a considerable period of time. Upon opening a little shop, thus gaining pleasant environment and company and a feeling of financial security, she obtained immediate and complete relief. In other words, through avoiding depression she could tolerate activity. Many analogous examples could be cited.

Exaggerated response to sensations is not common but is very definite in certain patients, including those sensitive to material agents and some to physical agents. Two pollen sensitive cases were so sensitive to the effect of pain as to make it practically impossible to administer treatment with a hypodermic needle. In one patient with urticaria dermatographia, hyperesthesia to pain was so marked that it was practically impossible for her to use a stiff brush. Occasionally pollen cases are so sensitive to heat that it is almost impossible to test them for reactions caused by heat. The heat of a lamp which to a normal individual would not be disagreeable may in them cause such a sensation of burning as to make them think they were being blistered. Others are occasionally sensitive to cold, in fact so much so that it may be difficult to test them for reactions caused by cold. The application of cold water at a temperature which would not annoy a normal individual may cause in them a sensation so intense as to interfere with the test. The same type of reactions occur in a few cases sensitive to friction on the skin. The sensation produced by a brush may be so marked as to interfere seriously with treatment of the case by a stiff brush.

The reactions described have all been observed in typical atopic cases, atopic in the strictest sense of the definition and also in physical cases. They are believed to be due to the overreactibility of certain groups of nerve cells in certain individuals to one or more specific mental or neurologic stimulants. The result is exaggerated response to a physical agent or mental impressions which would not seriously affect the normal individual or the average atopic case.

SUMMARY

A minority of atopic individuals and those having hay fever, asthma, or hives caused by sensitiveness to the action of physical agents, may show mental or neurologic peculiarities which might be described as hyperreactibility to impressions on subjects which concern them deeply and also hyperesthesia to certain physical agents.

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An egg and feather case who had travelled a considerable distance to consult me, regained almost perfect health through avoidance of egg and feathers. She came to the office once during a slight attack and upon being sent into an examination room, promptly placed her head between two pillows and remained there until she was asthmatic to such an extent that she required the attention of a nurse and a physician throughout the night. When asked why she did such a ridiculous thing as to smother herself in pillows when she knew she was sensitive to the feathers, she stated that when she entered the room she scarcely knew what she was doing.

In a previous paper I described at some length symptoms similar to those caused by pollen reactions except that they were caused by heat and in many instances by change from undue cold to an atmosphere which is relatively warm. The same reaction could be caused by the heat generated by either mental or physical activity and was often more pronounced if the patient had previously been at rest or was cold. I have observed a number of patients with identical reactions caused by mental activity and in many cases the reaction is augmented by a previous state of mental depression. A pronounced

nonspecific provocative causes. Indeed, I have had my share of cases in which from the cutaneous reaction I could show no evidence of protein sensitization. But this failure no more invalidates the hypothesis than does the failure to relieve all asthmatics or to always observe positive skin tests, disprove the allergic theory of asthma. In both eczema and asthma completely negative skin test cases may mean that our hypothesis is incomplete, that some cases are not dependent on allergy or that we have not tested with the proper proteins, or that the refinement of the test proteins has been insufficient, that our methods are not sufficiently delicate or that under certain circumstances an allergic individual will not give positive cutaneous reactions. I predict with confidence that as we learn more of clinical allergy all of these possibilities will be found to play parts of some significance and that increasing percentages of eczema as well as asthma and migraine and probably urticaria will be found to be unequivocally associated with allergy.

The contributions of O. Kiefe, Blaes, Peshkin and others on eczema have been sufficiently numerous to be convincing to those who have read them.

The frequent association between asthma and eczema is a matter of tradition. Coke⁴ found that of 1000 cases of asthma 18 per cent gave a history of eczema. Of 500 asthmatics found sensitive to foreign proteins 25 per cent gave a history of eczema. Of 250 asthmatics sensitive to food proteins, 37 per cent gave a history of eczema. Schloss and Holt¹ both remarked that a good proportion of infants or young children with bronchial asthma had had eczema during infancy. Blaes⁷ observed that victims of urticaria and angioneurotic edema and of asthma, where these were due to foods, nearly always gave a history of eczema in early life. Moro and Kolb⁸ found that, of 86 eczematous children, 23 per cent had asthma. Peshkin⁹ first made a comparative study in which not only the food proteins but also pollen dust and bacterial proteins were used. Of 100 asthmatic children 31 had had a dermatosis. Of these 31, 22 were protein sensitive. In Peshkin's experience eczema is usually based upon food sensitization, while asthma more frequently follows sensitization to inhalants. He suggests a prophylactic value in this observation in that an eczematous child found sensitive to food may then be tested to other proteins, particularly inhalants, in order to prevent the later onset of asthma.

It should be noted that among Peshkin's protein nonsensitive asthmatics the incidence of eczema (19 per cent) was almost as great as in those asthmatics who were found protein sensitive (22 per cent). In the allergic group eczema always preceded the onset of asthma, while angioneurotic edema was concurrent with or subsequent to the asthma and urticaria always began after the asthma. Asthmatics sensitive to foods who gave a history of cured eczema had no recurrence of the eczema on ingestion of those foods to which they were sensitive. These foods did, however, cause urticaria or angioneurotic edema and occasionally asthma. Asthmatics with persistent or recurrent eczema were not relieved of the skin condition by elimination of the foods. Their ingestion, however, served to increase the eczema. Those foods responsible for the greatest number of skin reactions in eczema were fish, meat, milk and eggs. Children with asthma, reacting to fish or meat, invariably gave a history of antecedent eczema.

tact the individual by some mechanism not completely understood maintains his allergic balance. An overdose of the same protein may disturb the balance with consequent precipitation of symptoms. One may daily ingest quantities of milk to which he is sensitized, maintaining allergic balance until in addition he eats, let us say bean, to which he is also sensitive. Neither alone would cause symptoms. Both together do. We may think of milk as being a predisposing cause and the bean protein as an exciting cause. While the predisposing cause is nearly always an allergen, usually one with which the individual comes in frequent or daily contact, the exciting cause may be either specifically allergenic, as just cited, or it may be nonspecific, nonallergic. The inciting cause may act locally as a mechanical irritation or it may act systemically, producing an alteration in the chemical equilibrium. Focal infection, endocrine disturbances, toxic absorption from constipation, acute infections, possibly also exhaustion and nervous reactions in this way serve as precipitating causes of allergic disease. This holds not alone in eczema but in asthma, migraine and the allied diseases. In my experience for example an eczematous type of dermatosis occasionally follows surgical operation. This usually occurs in the region of the elbows, at a place where mechanical irritation from rubbing on the sheet plays a part.

This interpretation of allergic disease as being sometimes the classical anaphylactic explosion, sometimes a disturbance of allergic equilibrium in which the provocative cause may be nonspecific, explains the good results obtained by other methods of treatment, and offers a suggestion as to alternative methods of treatment. Where two causes are at work in the production of one disease we can conceive that the removal of either one of these causes may result in a return to the balanced allergic state. To secure relief one may remove either the predisposing or the exciting cause. In eczema the patient may avoid the allergenic food or epidermal protein or rectify general dietary errors, he may remove the mechanical irritation and apply soothing ointments or he may clear up infectious foci.

The more scientific procedure would appear to be the removal in so far as possible of the cause. Ointments relieve but a symptom. Focal infection or constipation may actually be a cause. But where allergy plays a part I consider this to be the fundamental predisposing cause. The scientific method in these cases calls for the avoidance of those proteins to which the individual is sensitized. At the same time it requires the removal in so far as possible of the secondary provocative causes. The removal of all causes is the ideal to be striven toward but removal of any one may give relief from symptoms.

If it should develop that removal of contact with allergic proteins for a sufficient period actually allows the organism to overcome its sensitization, then allergic treatment is the method of choice. I have several cases on record in which the positive skin test has become negative during avoidance. In those cases where the skin test remains positive and yet the patient finds he can return to a general diet, I consider that he has returned to an allergic balance or equilibrium.

I do not wish to imply that I have relieved all cases of eczema by following a program of removing first the predisposing cause and if necessary the

nonspecific provocative causes. Indeed, I have had my share of cases in which from the cutaneous reaction I could show no evidence of protein sensitization. But this failure no more invalidates the hypothesis than does the failure to relieve all asthmatics or to always observe positive skin tests, disprove the allergic theory of asthma. In both eczema and asthma completely negative skin test cases may mean that our hypothesis is incomplete, that some cases are not dependent on allergy or that we have not tested with the proper proteins, or that the refinement of the test proteins has been insufficient, that our methods are not sufficiently delicate or that under certain circumstances an allergic individual will not give positive cutaneous reactions. I predict with confidence that as we learn more of clinical allergy all of these possibilities will be found to play parts of some significance and that increasing percentages of eczema as well as asthma and migraine and probably urticaria will be found to be unequivocally associated with allergy.

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Stuart and Farnham¹⁰ observed that in the first two or three years of life foods appear to be the primary factors in sensitization, that during the following two or three years there is about an equal division between food sensitization and inhalant sensitization and that following this there is a period of years in which inhalant reactions are much the more common, with at the same time an increasing number of cases failing to show any protein sensitization

They believe that sensitiveness to food proteins is most commonly encountered in early childhood and that there is a natural loss of sensitivity to these substances with increasing age, although the age at which such loss will take place is not predictable. Hypersensitivity to food proteins tends to be present at birth and may be lost, whereas they believe hypersensitivity to inhalant proteins to be acquired and more resistant to change

Ramirez¹¹ found that of 78 cases of eczema 38 yielded positive skin reactions to one or more proteins. White¹² reported 66 per cent positive reactions. Talbot¹³ observed 14 reactions to egg white and other food proteins in 16 eczema cases. Schloss⁵ found 77.4 per cent positive skin reactions in eczematous children under sixteen months of age and 41.6 per cent positive in children over sixteen months

Duke,¹⁴ in his monograph, classifies allergic eczema under the dermatoses and points out that these reactions may be from contact or may form a part of a general reaction. He points out that the skin test reaction may be very mild or uncertain

Kolmer¹⁵ remarks that up to the present time allergic eczema has been identified almost solely with food substances, that there can be no doubt that some cases are of an allergic nature, but not in as high proportion as commonly believed

The present report is based upon a consecutive series of 71 cases of eczema. Satisfactory follow-up work has been done in all but four. These four have been included but since I do not know the results of treatment they are grouped in the *no relief* section. Definite relief during the period of specific protein avoidance was obtained in 39 of these 71 cases, or 55 per cent. In two of these the improvement is recorded as amounting to about 50 per cent, in the remainder from 75 to 100 per cent. In all but two, food sensitization appeared to be the predominant factor. One of the two, a physician, was sensitive to novocaine and was relieved by avoidance. The other, an artist, sensitive to some foods, developed her eczema especially on contact with turpentine. A third, definitely an egg case, was also sensitive to wool, and wool likewise caused dermatitis. With the following exceptions all patients were treated by diet only and in no case were local ointments or other applications used. Three received peptone injections along with the diet. One of these also took mixed gland tablets. Two received x-ray treatment. Ray therapy gave relief but it was found in both cases that continuation on the diet was necessary for permanence of relief

This series differs from most eczema series in that it is made up chiefly of adults. Among the good result cases three were under two years of age, one was seventeen, two eighteen, three were between twenty and thirty and the

remainder were over thirty years of age. The oldest was sixty three. The duration of the illness ranged from six months to thirty eight years, most of them less than ten years.

The scratch method was used in all cases, with readings at the end of one half hour, from four to six hours and after twenty four hours.

A comparison of the good result series with the poor result series brings out some interesting features. There is evidence that protein sensitization plays a part in the poor result cases in spite of therapeutic failure.

The results are recorded as one plus, two plus, three plus, four plus, following the usual custom and as plus minus in those cases with borderline reactions and plus minus minus where the reaction as compared with the other scratches is but barely suggestive.

In the 39 good result series, which for brevity we shall call Group A, the immediate reactions were recorded as three plus in two cases, two plus in eleven, one plus in eleven and plus minus or plus minus minus in fifteen. In the poor result cases, or Group B there was one four plus reaction, one three plus reaction, eight two plus, six one plus and sixteen plus minus or plus minus minus. Definitely positive immediate reactions were obtained in 61.5 per cent of Group A and in 50 per cent of Group B.

In 39 of the entire series a history of other allergic diseases was sought. These included hay fever, asthma, urticaria and migraine. In Group A eight out of nineteen gave a positive past history for one or more of these other diseases and in Group B eight out of twenty. In Group A a positive family history for allergic diseases including eczema was obtained in eleven out of eighteen cases and in Group B fourteen out of nineteen cases. There was a family history of eczema in seven of eighteen in Group A and six of nineteen in Group B. A delayed positive reaction recorded as one plus or two plus was found in seventeen of thirty nine in Group A and seventeen of thirty two in Group B.

The parallelism between the two groups both with regard to character of reactions and personal and family history for allergic disease, is rather striking. I am inclined to believe that perseverance in the study of the poor result cases, possibly with the discovery of still other reacting proteins but particularly with the removal of nonspecific contributory factors, would have increased the percentage of good results. My eczema cases have been tested only with the food and epidermal proteins. Possibly some of the Group B cases would have shown a bacterial sensitization. Another factor which may in part be responsible for poor results is that of personal supervision. Where the individual is examined for another physician and the latter supervises treatment, his conception of the importance of complete diet restriction is often not the same as that of the allergist. I never feel as confident of good results in those cases where the tests are done on other doctors' patients. I had personal continued supervision over twenty two of my thirty nine Group A cases and but eleven of my thirty two Group B cases.

Turning now to a consideration of Group A alone,—those patients showing definite relief or marked improvement while on the dietary restrictions. In regard to the character of the reaction, I lay some stress on the importance

of reading the delayed reactions. The immediate reaction is usually the one of greatest importance but valuable information may often be gained from the four-hour and the twenty-four-hour readings. I have said that 61.5 per cent of Group A gave definite, positive half-hour reactions, 35 per cent gave delayed reactions, one plus or stronger. Often these positive delayed reactions were observed where the immediate reactions had already been clearly positive and here of course the evidence was merely confirmatory. But in seven of the thirty-nine the delayed reaction was recorded as relatively more intense than the immediate and here, of course the delayed reaction assumes diagnostic importance. In seven instances the diagnosis was based on the delayed reaction only, the immediate reaction being no stronger than plus minus. Four of these seven showed the strongest reaction at the end of from four to six hours and three at the end of twenty-four hours. The delayed reaction consists of a zone of erythema surrounding the scratch, somewhat resembling a beginning infection. It is not as pronounced a reaction as the immediate.

I would stress the importance of considering the very mild reaction. In six of the thirty-nine successful cases the reactions at all three readings were minimal, that is, not greater than plus minus. Diagnosis was made on the basis of borderline reactions to some one protein being observed at all three readings. The results of treatment would appear to justify this procedure. Recording borderline reactions is entirely rational, particularly when the scratch method is employed as we do not know exactly how much protein in solution has come in contact with the reacting tissues. Another point essential for success in the delayed reading is that the scratch be made sufficiently large. A three-eighths inch scratch appears ideal. With a one-eighth inch scratch insufficient protein may be applied. I have presented elsewhere my reasons for considering a mild reaction and the delayed reaction rather characteristic of chronic protein poisoning such as is usually the case in eczema. Time does not permit that I repeat them here.

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ALLERGY IN THE ETIOLOGY OF DISEASE*

By ALBERT H. ROWE, M.S., M.D., OAKLAND, CALIF.

PROTEIN sensitization or allergy is the cause of many symptoms other than those of hay fever and asthma. Sensitization to bacterial proteins is now known to produce most of the symptoms and pathology of infections such as tuberculosis and scarlet fever. Many types of dermatitis, urticaria, abdominal pains, and indigestion, as well as certain cases of migraine and epilepsy, are due to allergy. Allergy undoubtedly enters into the symptomatology of other acute and chronic diseases, and it behooves the medical profession to recognize its importance, and watch the unfolding of the important role it plays in the production of disease.

GENERAL CONSIDERATIONS

Skin testing, as introduced by Schloss in 1912, has made possible the rapid development of the subject of allergy. The physician must realize, however, that skin tests are not always positive on patients clinically sensitive to a given protein. This is especially true in food sensitization, as originally emphasized by Schloss. Negative skin reactions are also present in patients sensitive to animal emanation, dust, and pollen proteins.

Inability to demonstrate by skin testing all sensitizations, at one or several sittings, necessitates the taking of a detailed allergic history, a willingness on the physician's part to retest the patient several times if necessary, and a mastery of the literature and technique of allergy by the physician who undertakes its treatment.

I am a strong advocate of the cutaneous "scratch" test. The intradermal method is used only in patients suspected of pollen, animal emanation, dust, and other miscellaneous sensitizations, who give negative cutaneous reactions. Fatal results occasionally occur with the intradermal test, which offers ample reason for its abandonment for general use. I am sure that the food and animal emanation proteins of Arlington and Squibb are quite dependable and probably those of other commercial houses are efficient. The best way is to prepare one's own proteins from local products, and to use only such proteins as give positive reactions in patients known to be sensitive to those proteins. In my work, we have available nearly 200 such privately prepared proteins as well as certain commercial ones, and over 200 privately collected California pollens.

A thorough analysis of the patient suspected of protein sensitization should include testing with the proteins of all foods and condiments, animal emanations, local tree grass weed, and shrub pollens, a number of active stock house and hay dust extracts, orris root, pyrethrum, and other miscellaneous substances. One should likewise be prepared to make an extract of any

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suspected dust or other substance to which the patient might be sensitive. With such study, most of the active as well as potential sensitizations will be discovered.

Many problems of allergy may be worked out successfully by any physician who will give it his earnest study and unprejudiced attention, and who will endeavor to be as thorough and painstaking as possible. Such an attitude is necessary if good results are to be obtained, and if the subject of allergy is not to be discredited by patients and physicians.

ABDOMINAL ALLERGY

Abdominal symptoms due to food sensitization are very common in childhood. It is well recognized that certain children cannot take milk, eggs, or other foods, without vomiting, abdominal cramps, diarrhea, or even shock.

Many children difficult to feed are probably allergic individuals. Food dislikes and disagreements in childhood, as well as in adult life, should first be thought of as due to allergy rather than to notions and whims, in justice to the patient. Where allergy is suspected, children should not be forced to eat food which is definitely distasteful to them. Food sensitization may also explain the varying appetites that children evince, which may be protective in nature.

Abdominal allergy occurs frequently in adult life, just as it does in childhood. The adult who has allergic reactions to shell fish, berries, or fruits, is frequently found. That many other foods, especially common ones, can produce abdominal allergy in the adult, is not generally recognized.

The following history presents a marked case of abdominal allergy.

A man of 45 years was referred because of asthma and hay fever. He stated that he was born with an egg idiosyncrasy which has steadily grown worse, so that he cannot take the smallest bit of egg without indigestion. Marshmallows, centers of candy, or ice cream made with egg, cause marked symptoms. He first notices itching of the back of tongue and pharynx, with swelling and burning of the mucosa. Forced vomiting gives immediate relief, but if he doesn't vomit, indigestion consisting of a swollen sensation, burning, and some gas, will persist in the abdomen for several days. Egg on the skin causes swelling. Egg shampoo at one time caused hive like swellings all over his scalp, forehead and eyes, and egg when eaten results in a dry cough that lasts for days.

This is an example of definite abdominal allergy in adult life. It emphasizes the fact that such allergy is not always outgrown in childhood, and suggests that even if amelioration does occur, certain mild residual manifestations of a food sensitization may persist in later years and be the cause of indefinite abdominal distress. However, abdominal allergy due to food sensitization may develop out of a clear sky in adult life.

Another instance of abdominal allergy in adult life follows.

Mrs. A. P., aged 26, was referred because of indigestion, as well as for asthma and hay fever. Her physical examination had been negative, and her physician suspected that the indigestion was allergic in type.

Her nose had been constantly congested since the onset of her asthma ten months before, and daily sneezing had occurred. Dusts and powders had not aggravated her symptoms. Sojourns at Los Gatos, and finally at Palm Springs, had given no relief. All foods seemed to agree with her. She always had had definite indigestion, however, consisting of

soreness, bloating, and fullness in the abdomen. Skin testing with 235 proteins gave no positive reactions.

I assumed she was food sensitive in spite of negative skin reactions, and she was placed on a limited though balanced diet, which absolutely excluded all common foods. This diet has gradually been expanded, but she cannot take wheat, or egg, without abdominal distress. Without wheat and egg she is entirely free of all abdominal, as well as hay fever and asthma, symptoms. This case demonstrates that food sensitization may cause abdominal symptoms, as well as perennial hay fever and asthma, in the same individual.

Certain atypical digestive histories in adults may likewise be due to food allergy. Such possibility should be kept in mind if there is a family history of allergy, or if there are definite allergic manifestations in the patient.

The following is a very interesting history of this type.

B. Y., aged 47, complained for three days of severe abdominal pain, especially in the lower left quadrant, associated with dullaching through the midabdomen and in the left lumbar region. It first suggested kidney colic or intestinal pain due to colitis or adhesions. On three occasions during the year, he had had pain and indigestion after eating chicken. The fact that strawberries had caused a terrific rash and that bananas had always upset him made me suspicious of allergy. Careful physical examination including a complete roentgen ray study, was negative, except for a generalized psoriasis. Skin tests gave delayed reactions to four wheat proteins, to banana, strawberry, fig, mushroom and tomato proteins, and a positive reaction to chicken protein. He has excluded these foods from his diet for two years, and his symptoms have entirely disappeared. He has tried eating wheat, chicken and mushrooms on several occasions, and the abdominal pain and indigestion promptly returned. Guinea hen meat even produced trouble. His psoriasis has likewise been greatly benefited.

It is probable that abdominal allergy is more common in both mild and severe forms than is generally appreciated. Patients having hay fever and asthma commonly have food sensitizations responsible for indigestion. Lentz, of Brooklyn, believes that many appendicectomies are removed because of congestion due to food sensitization, rather than to infection. The physician, therefore, must be sure that food dislikes and disagreements of which patients complain are not due to food sensitization before they are dismissed as being of neurotic origin, and he must keep allergy in mind in the analysis of gastrointestinal symptoms.

ANGIONEUROTIC EDEMA

It has been assumed for several years that angioneurotic edema is due to protein sensitization. Piness, however, has recently stated that angioneurotic edema, characterized by nonitching, painless swellings, is not due to protein sensitization, whereas giant urticaria is due to allergy. I have studied cases, however, which seem to be of the type of angioneurotic edema, and which have been relieved by treatment based on allergy.

Mrs. C. O., aged 52, has had four attacks of acute swellings of face and hands. These swellings did not itch and became so great that eyes closed and lips puffed out. Tongue became swollen so that she couldn't talk, swallowing became difficult, and severe depression resulted. Generalized welts over the body usually appeared about two hours after the onset, with relief to the extreme swelling of the face. Itching of palms of hands and palate occurred frequently and did not result in angioneurotic edema if a large dose of citrate of magnesium was taken at the first sign of such itching.

For years before angioneurotic edema developed, she had excruciating attacks of abdominal colic, recurring at least every month, which were undoubtedly due to allergic congestion in her gastrointestinal tract

She continued to have belching and indefinite indigestion for years, which she has ascribed to certain foods, such as cabbage, asparagus, onion, lettuce, and sweet potato. Three plus reactions were obtained to cabbage, celery, and asparagus, and one plus reactions to potato, lettuce, parsnip, onion, wheat globulin, rice, egg, and banana proteins. On a diet excluding these foods, her indigestion has been definitely relieved, and she has not been threatened with attacks of angioneurotic edema. It is to be noted that skin reactions were obtained to the foods which she knew disagreed with her.

Miss C. L., aged 17, for six months had sudden nonitching swellings on fingers, palms, lips, and around eyes, but there was no indigestion. Physical examination, including a gastrointestinal roentgen ray study, was negative. Skin testing gave delayed reactions to wheat, and for two years there has been no recurrence of swellings since she has been on a wheat free diet. Desensitization with wheat protein solution was carried out, without satisfactory results. If she eats wheat, she immediately notices a marked flushing all over the body, and has a return of her swellings. This case demonstrates the importance of wheat as a cause for sensitization, the importance of delayed reactions, and the rôle that sensitization plays in the causation of angioneurotic edema.

It may be that certain cases of angioneurotic edema are not due to allergy. I believe, however, that each case challenges the physician from the point of view of protein sensitization and should be studied over a period of weeks before allergy is dismissed as a cause.

URTICARIA

Hives are most frequent in childhood, and are a constant problem for the pediatrician. Dr. Clifford Sweet thinks that since sun-baths and well-balanced diets have been insisted on in his patients, hives are less frequent. He also believes that infections at times are either directly responsible for hives or precipitate potential outbreaks.

Undoubtedly a high percentage of urticaria is due to food sensitization. We have assured ourselves of this through the study and dietary control of a large number of cases.

A typical case of urticaria in childhood is here summarized.

C. S., aged 4, had had hives continually for three years. These had been present nearly every day, and in spite of much medical advice, no relief had been obtained. Skin tests with all common foods were negative. In spite of this, a diet excluding all common foods was first prescribed with immediate relief to the patient. He has been entirely free for one year, and is now on a diet in which the chief characteristic is freedom from wheat and eggs. The mother had tried to exclude eggs from the child's diet before, but had given certain foods which contained small amounts of egg. This patient has failed to give positive skin reactions to wheat and eggs on several retestings, and relief was obtained on the diets prescribed on the basis of experience. This emphasizes the fact that skin testing must be used by the allergist only as an aid in the treatment of his cases.

The case of Miss M. L., aged 13, is of interest. She had had hives intermittently for six months, and every night for five weeks. These hives occurred especially on her back and abdomen, and on areas where legs touch each other. For some years, she had known that her lips and face became swollen when she ate tuna fish. Skin reactions were positive to wheat globulin, egg, and to tuna fish, halibut, and codfish. A wheat, fish, and egg free diet has given complete relief, except when she ate a lady finger, and again when she took a little ice cream made with egg.

Urticaria in adult life is often chronic and distressing. Sometimes, relief may be obtained by regulation of diet based on allergic investigations. The type of allergy responsible for many cases which give no skin reactions and get no relief even from starvation is difficult to ascertain. Treatment with Alpine light, with intravenous calcium chloride and peptone injections, has been disappointing. Absorption of toxins from the intestinal tract, or from some metabolic imbalance, or from a focus of infection, may be considered possible causes. A recent case received nearly complete relief from urticaria of six years' duration by the treatment of a duodenal ulcer with a modified Sippy regime. Infection in the ulcer may have caused the malady.

I feel that much urticaria is due to food allergy. Bacterial allergy and certain types of allergy which are as yet poorly understood, may be at the basis of the etiology of some urticaria.

DERMATITIS

Skin irritation due to protein sensitization may result in generalized itching—with or without a rash—or in a definite eczema. A sensitized skin may come in contact with proteins to which it is sensitive, either by way of blood carrying food or pollen proteins or by actual contact. Contact dermatitis is usually present on circumscribed areas, while dermatitis due to food is apt to be diffuse. This rule, however, does not hold in all cases.

A Chinese man, aged 26, had had a constant generalized itching all over his body for five years. There was a very fine eruption present. Skin testing with all types of protein gave slight reactions to wheat and milk as well as to several other less common foods. A diet based on these reactions immediately controlled the itching and there had been no return for three months when he was last seen.

Mr S. A., aged 55, had had generalized itching and dryness of arms, back, and legs, for two years, with recent lesions in circumscribed areas. Mushrooms gave violent abdominal pain. Skin reactions to all foods were negative. He was placed empirically on a limited diet, excluding common foods. Relief was obtained and his diet was gradually enlarged, so that now wheat, egg, and most fresh fruits are alone excluded.

As one would expect, our statistics show that people are most often sensitive to common foods. Hence, empiric diets can be prescribed on the basis of average skin reactions, where food sensitization is suspected and skin reactions are negative. Such diets often yield excellent results.

Many interesting cases of contact dermatitis are encountered in the practice of allergy. Brief mention will be made of several cases of this type.

Miss E., aged 58, had had an itching swelling of her face and neck for two years, which had resisted all therapy. Skin tests showed a marked reaction toorris root, and discontinuance of powders and cosmetics gave immediate relief.

Mrs. F., aged 25, had had an eczema on her hands for twelve years. She gave positive reactions to several wheat proteins, and the eczema disappeared when she stopped handling wheat flour. Dr. Rogers and I reported a dermatitis confined to the hands and forearms, due to contact with mohair.

A child of three years had had a marked exuding eczema on arms, legs, and face for one and one-half years. Skin reactions were obtained to horse dander, milk, and wheat proteins. The child's father kept twenty horses in a barn near the house. When the child's environment was kept free from horse dander, and the diet from milk and wheat,

the eczema disappeared. The mother put wheat and milk back into the diet after relief had been obtained, with immediate return of the eczema.

Mr. R., aged 38, had had an itching eczema on face, neck, arms, and legs, for three years, appearing each spring and becoming extremely severe by September. On leaving his chicken ranch, he would obtain immediate improvement. Skin testing gave three plus reactions to *Artemisia vulgaris* and *biennis*, and to *ambrosia psilostachya*, and to flaxseed, which was in the chicken mash he was using.

An interesting type of skin allergy is present in a woman of 41, whose skin blisters and peels off whenever she handles mountain trout. The skin around her mouth, and the mucous membrane of her lips and mouth likewise peel off and violent nausea, vomiting, and abdominal cramps occur, if she eats trout. Another unusual skin sensitization was found in a child, causing burning and excoriation of the skin of thighs and cheeks, which came in contact with urine or tears. The mother, one of our hay fever patients, had a similar type of skin irritation. No relief had been obtained for the condition in her child. Skin reactions were obtained to string beans, spinach, squash, strawberries, and wheat. The mother has gradually determined that of these proteins giving positive reactions, string beans and wheat cause the chafing.

MIGRAINE AND EPILEPSY

Both migraine and epilepsy have been included by many writers in the diseases due to protein sensitization. Joseph Capps and Joseph L. Miller of Chicago have written on both these diseases from the allergic viewpoint. I have observed and treated several cases of migraine and epilepsy that were benefited by treatment based on allergic studies, and I believe that allergy should be considered in all such cases where there is an allergic background in the patient, or in his family history.

Mrs. P. S., aged 51, had had severe headaches all her life, recurring every few days, and lasting 2 to 4 days at a time. She had never been entirely free from dull aching in her head. In spite of repeated examinations and treatment, no relief had ever been obtained. Thorough physical examination was negative, except for a rectal stricture. Stomach analysis, Wassermann, blood count, urine analysis, stool examinations, and roentgen ray examination of chest and gastrointestinal tract, were all normal. Protein sensitization tests showed delayed reactions to wheat, fig, prune, halibut, sole, celery, onion, pea, tomato, and positive (one plus) to asparagus, lettuce, lima beans, potato, and mushroom. On a diet based on these reactions, she has been entirely free from her headaches for over three years. Occasionally, she eats a salad containing a vegetable to which she gave a reaction, and a slight headache results. Dizziness, morning nausea, and a severe numbness in the left arm have entirely disappeared.

I have four other patients with migraine who have obtained great relief on diets based on skin tests. It is impossible to say what percentage of migraine is due to allergy. Sensitization to bacterial proteins, or to other foreign proteins of unknown nature, could well cause the trouble. An intensive study should be made of a large number of causes of migraine from the sensitization viewpoint. Capps has obtained good results with peptone injections in migraine, without dietary restrictions. J. L. Miller recently told me, however, that such relief was short lived and that the peptone had to be reinjected.

EPILEPSY

Ward in 1922, Howell in 1923, Wallis and Nicol in 1923, and J. L. Miller in 1924, discussed epilepsy from the viewpoint of sensitization to food. Wallis and Nicol found a considerable number out of 122 epileptics benefited by diet.

adjustment based on skin testing and oral administration of peptone. It is not improbable that improvement obtained with high ketogenic diets is in part due to the removal of foods such as wheat and milk, to which the patient may be sensitive. We have several records of asthmatics who have had convulsions in childhood, which suggests that many such seizures in children are due to protein sensitization.

One record of epilepsy entirely controlled by pollen therapy, given for a pollen asthma, is unique, and will be briefly summarized.

F V, aged 4, had had continuous asthma for two years, especially severe in summer and fall. With the onset of asthma, severe right temporal headaches occurred every 10 to 14 days culminating in convulsive attacks followed by delirium and unconsciousness for 8 to 10 hours. For several months, he had had typical petit mal attacks three or four times a day, and continuous asthma with abundant expectoration. He had had constant insomnia and marked irritability. Skin testing gave positive reactions to twelve spring and fall pollens. Desensitization to these pollens was given throughout the entire first two years, with marked relief of the asthma, and, what was not expected, control of the epilepsy after the first two months of therapy. Pollen treatment has now been given for four years, and the asthma has been entirely controlled except for an occasional attack of wheezing when the pollen is especially abundant. The epilepsy has been entirely absent, but slight headaches with irritability have occurred occasionally.

A R, aged 11, had had petit mal attacks, for about 6 years. She could never be alone, and had them in school as well as home. Her mother had had hay fever. Skin tests gave positive reactions to cat, horse, and rabbit hair proteins. Desensitization to horse hair and removal of her hair mattress has resulted in complete relief from her attacks for over two years.

These two cases are not duplicated in the literature—the first where epilepsy was controlled by pollen desensitization, and the second where petit mal attacks disappeared by control of animal emanation sensitizations.

Allergy undoubtedly is the cause of certain cases of epilepsy, especially in children. The percentage of cases due to protein sensitization may not be large, but in the investigation of epileptics, allergy should certainly be seriously considered.

HAY FEVER

As I continue, from year to year, my treatment of patients with seasonal hay fever, certain things impress themselves on my mind. Pollen therapy needs to be specific and several antigens, each containing three or four separate pollens, may be necessary during a season. Many patients are sensitive to pollens of all seasons, this necessitates changing from one antigen to another before the new pollens are in the air. The preparation of special pollen antigens for each patient is usually necessary for specific therapy.

Results are generally very satisfactory and improve as the treatment is repeated year after year. Some few patients have gone through a season without treatment, and skin reactions to pollen have disappeared in several cases after three or four years of therapy.

Seasonal foods and dusts, rather than pollen, may cause seasonal hay fever. Most patients with hay fever should be thoroughly tested with all types of proteins, as well as with pollens, to determine active or potential sensitizations other than pollen. A recent patient, with symptoms only during May

and June, gave no reactions to any pollens, but did react strongly to a stock hay-dust extract. She dated her hay fever every year from the cutting of the hay on a nearby hill. Relief was obtained by desensitization to this hay-dust extract.

It is necessary to remember that insect pollinated flowers, trees, and shrubs, can produce hay fever. The patients have to be in closer contact with the source of such pollen, however, than with wind-borne pollens, which may blow 15 to 20 miles. Acacia pollen sensitization has been encountered several times in the last year, and hay fever due to pollen of cultivated flowers is, of course, very common. Definite seasonal hay fever, however, is in nearly every case due to wind-borne pollen.

Those physicians who undertake the treatment of pollen hay fever or asthma should become thoroughly acquainted with the complete botanical flora of the regions of the state from which their patients come. For a very intimate knowledge of the pollens of any one district, the study of pollen plates is most helpful. I have made, through the help of my botanist, Miss Weisendanger, and office assistants, counts of pollen plates two to three times a week at from 5 to 9 different locations from Richmond to San Leandro, in the East Bay Cities. This count has extended over a period of one year. This has given me an accurate method of gauging our pollen therapy in an area where pollen is in the air throughout the year.

PERENNIAL HAY FEVER

Certain cases of hay fever, lasting throughout the year here in California, may be due to pollen sensitization alone. Other patients may be sensitive to orris root, which is an ingredient of nearly all face powders, cosmetics, and tooth powders. Sneezing throughout the year, especially in damp weather, is often due to sensitization to animal emanation dusts, particularly to that arising from feathers, wool, or horse hair or dander. House dust is a very frequent cause of nasal irritation, congestion, and of sneezing. Some patients react to house dust extracts alone, and have relief from therapy based on such reactions. Perennial hay fever may also be due to food sensitization, though I have never seen it as a sole cause. There are certain patients who have constant congestion of the nose, without other allergic manifestations, and without any skin reactions. Such patients very often have chronic infection of antrums or other sinuses, and should receive surgical attention.

SURGERY

A conservative attitude in regard to surgery in the treatment of perennial hay fever and also asthma should be emphasized at this point. All such cases should be placed under indicated allergic treatment. If definite signs of sinusitis persist after such control is instituted, or acute symptoms from sinusitis occur at any time, surgery should be done for relief of infection and better drainage.

I have noticed in my experience with the treatment of hay fever and asthma, that where surgery has been performed, especially of an extensive nature, the allergy is often difficult to control. A patient referred twelve

months ago because of perennial hay fever had had seven different nasal operations. Marked purulent discharge was still present, and the nose specialist had in mind further surgery. We found definite pollen, orris root, and food sensitizations, and marked relief has been obtained through desensitization. Where such marked congestion of mucous membranes due to sensitization is uncontrolled, I cannot imagine that surgery can be done without an extension of the infected area. For the control of either hay fever or asthma, I see no justification in cutting off turbinates or straightening septums, or taking out tonsils, before a thorough allergic study and indicated treatment based on allergy has been done.

BRONCHIAL ASTHMA

I have left until last my remarks on this common and distressing result of protein sensitization. My ideas concerning the etiology have not changed materially in the last two years. Sensitization to animal emanation, pollen, food, dust, orris root, bacterial, and other miscellaneous proteins, follow in the order of frequency.

During the last two years, I have given much attention to the question of house dust in the etiology of asthma. In a series of 162 new patients with asthma, seen in private practice during 1925,* 45 per cent gave one or more positive reactions to stock house dust solutions. This demonstrates a most important factor in the etiology of asthma, and emphasizes the importance of its control.

I still believe that infection as a cause for bronchial asthma rarely occurs without some other type of sensitization, especially to dust or animal emanation or pollen proteins. Occasionally, bacterial sensitization alone seems to explain asthma, but only on rare occasions.

The following type of history is frequently obtained, and without careful allergic studies, can misguide the physician into the diagnosis of bacterial sensitization.

A child of three and one half years had pneumonia at 6 months, frequent bronchitis and colds during the next year, and for the last one and one half years bronchial asthma recurring every 1 to 2 months, associated with high temperature and vomiting. He had sneezed a great deal, especially on arising, his nose itched, and he had coughed much during the night. Skin reactions gave two plus sensitizations to spinach tomato, and to stock house dust No. 13, one plus reactions to sheep wool, cat hair, stock house dusts No. 4, 5, 25, 27, orange, and marked delayed reactions to all wheat proteins. This child had had antogonous and stock vaccine therapy through another physician for several months, without relief. The child was placed in an environment free from wool and feathers, and on a diet indicated by his skin reactions and history, and the daily sneezing and coughing immediately ceased and no asthma has recurred in a period of four months.

Thus this asthma was found to be due to animal emanation, dusts, and food proteins, though the history would indicate the possibility of infection being at the basis of the symptoms.

Many irritative, persistent coughs are also due to allergy. Such may occur without asthmatic or nasal symptoms. A child of three years seen in my asthma clinic in the Health Center had had a constant cough, especially

*House Dust in the Etiology of Bronchial Asthma and Hay Fever Arch. Internal Med. April 1927 xxxix, 498-507.

at night, for two years. Positive skin reactions were obtained to feather and milk proteins, and the cough rapidly disappeared on indicated therapy.

A child of 7½ years had had a nearly continuous cough for six years. Tonsils and adenoids had been taken out, without relief. Removal of wool dust from the child's environment has stopped the cough for over a year. These cases point out the necessity of considering protein sensitization in all patients with repeated colds or bronchitis, whose examination is essentially negative.

In order that the best results may be obtained in the treatment of asthma, patients and physicians must remember certain facts. Pollen sensitive patients should be desensitized preseasonally, and in some cases during the season as well. A hypodermic may even have to be given every week for eight to ten months to control the very sensitive pollen asthmatic. If such simple therapy, however, controls an invaliding asthma, little argument in favor of it is necessary.

Sensitization in asthmatics is usually multiple. A patient may be sensitive to several animal emanation proteins, as well as to several pollen, food, or other miscellaneous proteins. Such sensitization at times must be worked out from the history, when skin tests do not show all the sensitizations present. Because of these facts, to do justice to the patient and to the subject of allergy itself, the physician must be willing to devote much energy and thought to the diagnosis and treatment of bronchial asthma.

It must be kept in mind that a cardiac or other systemic complication may be exaggerating the asthma. Finally, the patient must realize that asthma can rarely be entirely cured, that the hope of the allergist is to lay out a method of control which, if followed conscientiously from year to year, will lessen the asthmatic problem from 75 to 100 per cent, and will finally diminish its ease of recurrence.

CONCLUSION

The medical profession must recognize the large part allergy plays in the causation of disease. Physicians must discourage any skepticism about allergy as a cause for its many manifestations, since this merely retards the necessary recognition of this important and far-reaching etiologic factor in disease.

ALLERGY AND EPILEPSY ANALYSIS OF ONE HUNDRED CASES*

By RALPH H SPANGLER, M D, PHILADELPHIA, PA

WE ARE just beginning to learn a little about the relation between epilepsy and allergy. The etiology of essential or so called idiopathic epilepsy points more and more, as recent investigations are recorded, to the probability that the immediate cause of the epileptic convulsion arises from a disturbance of metabolism. At any rate, the fact that allergy results in attacks of epilepsy in some individuals cannot be denied and certainly is worthy of consideration when we come to the treatment of this little understood and perplexing "symptom complex of disturbed metabolism"—the epileptic individual.

The present report is based on a review and summary of my last 100 consecutive adult cases of epilepsy with especial reference to allergic manifestations in the patient and the ancestors. Among the last one hundred and eleven patients in private practice who consulted me for convulsive seizures, eleven were children under fourteen years of age, forty three were females in whom menstruation had been established, and fifty seven were male adults.

In the eleven children (six girls and five boys) there were a number of interesting allergic findings, both in the patient and the ancestors, which undoubtedly point to their being potentially allergic epilepsy. These cases, however, have not been included in this tabulation since a definite diagnosis of epilepsy before the age of puberty, with our present knowledge, is more or less uncertain and can be made only after excluding spasmodic with so called reflex convulsions arising in connection with teething, polyps, phimosis, worms, fright, emotional disturbances, etc., and after excluding convulsions associated with acute infectious diseases, congenital abnormalities, syphilis and organic brain lesions.

This series of 100 cases, therefore, includes consecutive adult patients, whose histories are briefly summarized in Tables I to IV. Table I summarizes the history of 43 adult female epileptics.

Table II summarizes the history of 57 adult male epileptics.

Table III summarizes deductions of Tables I and II.

Table IV represents hereditary deductions from Tables I and II and is a summary of 100 (43 female and 57 male) adult cases of epilepsy showing the incidence of allergic manifestations in the patient, in the brothers and sisters, and in the ancestors (parents, grandparents, uncles and aunts).

IMMUNOLOGIC EVIDENCE OF ALLERGY IN EPILEPSY

There is certain immunologic evidence that points to the anaphylactic nature in some cases of epilepsy. We know that anaphylactic shock can produce

*Read at the Fifth Annual Meeting of the American Association for the Study of Allergy, Washington D C May 17 1927.

TABLE I
TABLE SUMMARIZING THE HISTORY OF 13 ADULT FEMALE EPILEPTICS

CASE NO	AGE	MARRIED OR SINGLE	ORDER OF BIRTH	BIRTH, NORMAL OR ABNORMAL	BUST OF BOTTLE FED	CONVULSIONS IN INFANCY	AGE WHEN ESTABLISHED	AGE WHEN DIAGNOSED	IN PATIENT	ALLERGIC MANIFESTATIONS		IN ANCESTORS AND FAMILY
1	12	M	3	Nor	Br	No	16	32	<i>Itves</i> when child	<i>Migraine</i> until 30	<i>Asthma</i> —Mother <i>Hay fever</i> and <i>mild asthma</i> —Father	
2	18	S	2	Nor	Br	No	13.5	16	<i>Eczema</i> and <i>severe migraine</i>		<i>Eczema</i> —Mother	
3	20	S	1	Nor	Br	No	15	10	<i>Itves</i> tenth to fourteenth year		<i>Migraine</i> —Mother and maternal grandfather	
4	19	S	1	Nor	Br	No	14	12	<i>Itves</i> (Scarlet at 6)		<i>Asthma</i> —Maternal grandfather <i>Convulsions</i> —Aunt in childhood, Maternal <i>Epilepsy</i> —Maternal first cousin	
5	16	S	5	Nor	B and B	Yes	12	7	<i>Enteritis</i> second summer with <i>convulsions</i>		<i>Migraine</i> —Sister <i>Asthma</i> —Maternal grandmother	
6	28	S	5	Nor	Br	No	14	18	<i>Petit Mal</i> 3 to 18		<i>Migraine</i> —Mother	
7	20	S	5	Nor	B and B	Yes	13	17	<i>Migraine</i> 12 to 18 <i>Digestive Disturb</i> and <i>convulsions</i> second yr <i>Itves</i> and <i>Eczema</i> (scalp)		<i>Asthma</i> —Mother <i>Migraine</i> —Maternal grandmother	
8	18	S	5	Nor	Br	No	13	15	<i>Petit Mal</i> since 9 <i>Eczema</i> —infant		<i>Migraine</i> and <i>Rheumatism</i> —Mother	
9	55	M	1	Nor	Br	No	12	19	Scarlet fever with convulsions at 8 years		<i>Migraine</i> —Father <i>Asthma</i> —Father's sister's two children <i>Asthma</i> —Mother and brother <i>Convulsions</i> —Sister (died of) <i>Hay fever</i> —Patient's daughter None obtainable	
10	18	S	3	Nor	Br	No	13	14	(Scarlet fever at 3) (<i>Convulsions</i> after tonsillectomy at 9)		<i>Asthma</i> —Paternal grandfather	
11	17	S	5	Nor	Br	No	14	14	<i>Petit Mal</i> started at 12		<i>Migraine</i> —Mother	
12	37	M	1	Nor	Br	No	15	30	First <i>convulsion</i> when pregnant seventh time Then none until pregnant three years later		<i>Migraine</i> —Mother	

TABLE I—CONT'D

13	25	S	4	Nor	Br	No	12	13	None	
14	21	S	3	Nor	Br	No	13	19	After weaning at 3 months digestive disturb ance and eczema Migraine when menstruation established	<i>Asthma</i> —Father Convulsions—Brother (16) and sister (18) <i>Hives</i> and eczema—Sister <i>Bilious spells</i> —Maternal grandmother and brother <i>Eczema</i> —Father and paternal grandmother <i>Hives</i> —Mother and brother <i>Spasms</i> —Sister when teething <i>Asthma</i> and <i>Hay fever</i> —Maternal uncle Hypersensitivity to certain foods—Sister <i>Migraine</i> —Father and his relatives <i>Hives</i> —Father his brother and sister and half brother of patient <i>Asthma</i> —Maternal uncle <i>Migraine</i> —Father and paternal grandmother <i>Asthma</i> —Son of patient Convulsions—Sister <i>Migraine</i> —Daughter <i>Hay fever</i> —Two maternal aunts <i>Hives</i> —Two maternal and maternal aunt <i>Migraine</i> —Mother and maternal aunt <i>Hay fever</i> —Brother <i>Hives</i> —Mother Convulsions—Maternal grandfather <i>Migraine</i> —Mother <i>Asthma</i> —Paternal grandfather <i>Migraine</i> —Father and paternal grandmother <i>Asthma</i> —Maternal grandmother <i>Asthma</i> —Mother <i>Asthma</i> —Mother <i>Migraine</i> —Sister <i>Migraine</i> —Mother and maternal grandmother Urticaria—Older sister, and her son <i>Asthma</i> <i>Asthma</i> —Father <i>Migraine</i> —Mother <i>Migraine</i> —Mother until menopause
15	22	S	2	Nor	Br	No	18	19	<i>Hives</i> when a girl <i>Migraine</i> since 15	
16	23	M	2	Nor	Br	No	12	16	<i>Migraine</i> since 9	
17	24	S	1	Nor	Br	No	13	20	<i>Hives</i> after strawberries second year (Scarlet fever at 5) <i>Eczema</i>	
18	15	S	1	Nor	Br	No	12	13	<i>Eczema</i>	
19	59	M	1	Nor	Bot	No	15	38	None	
20	16	S	3	In	Bot	Yes	14	13	Digestive disturb and const in infancy <i>Eruptions</i> and <i>hives</i>	
21	26	S	2	Nor	Bot	No	14	23	(Scarlet fever at 10) <i>Etioides diarrhea</i>	
22	30	M	4	Nor	Br	No	14	19	<i>Eczema</i> —eczema <i>Migraine</i> since menstruation at <i>Eczema</i>	
23	16	S	4	In	Bot	No	13	13	<i>Eczema</i>	
24	21	S	7	Nor	Br	No	16	16	<i>Migraine</i> since 10 years <i>Eruptions</i> during hay fever season <i>Hay fever</i>	
25	28	M	2	Nor	Br	No	11	26	<i>Hives</i> first convulsion at 5 years (Scarlet fever at 11 years) <i>Hives</i>	
26	28	M	8	In	Br	No	13	18	<i>Eruptions</i> when school girl <i>Petit Mal</i> 12	
27	25	M	1	In	Br	Yes (1)	14	20	Convulsions—teething <i>Petit Mal</i> at 11 (perfume factory)	
28	32	M	2	Nor	B and B	Yes	11	20		

*Wassermann negative.

TABLE I—Cont'd

CASE NO	AGE	MARRIED OR SINGLE	ORDER OF BIRTH	BIRTH, NORMAL OR INSTRUMENTAL	BREAST OR BOTTLE FED	CONVULSIONS IN INFANCY	AGE MENSTRUATION ESTABLISHED	AGE EPILEPSY DIAGNOSED	ALLERGIC MANIFESTATIONS	
									IN PATIENT	IN ANCESTORS AND FAMILY
29	20	S	5	Nor	Br	No	14	15	None	Migraine—Father
30	24	S	3	Nor	Bot	No	15	22	Convulsions at 8 years	Convulsions—Maternal grandmother
31	24	S	4	In	Bot	Yes	15	16	Petit Mal at 18 years	Convulsions—Maternal aunt
32	21	S	1	Diff	Bot	Yes	15	13	Convulsions when teething	Hives—Father for years
33	27	M	1	In	Br	No	14	19	Petit Mal at 14—Migraine since 16	None obtainable
34	15	S	2	Nor	Bot	No	11	19	Digestive upsets with convulsions when infant	Migraine—Mother
									Eczema and migraine	Convulsions—Mother, maternal grandfather and uncles
35	17	S	6	In	Bot	Yes	14	15	None	Hives—Mother
36	43	S	3	Nor	Br	No	15	15	Convulsions when teething	Asthma—Paternal grandfather
37	27	S	5	Nor	B and B	Yes	15	25	Petit Mal at 13	Migraine—Mother and maternal grandmother
									Second summer on bottle	Spasms—Sister (teething)
									Convulsions with digestive "upsets"	Convulsions—Father
38	25	S	3	Nor	Br	No	16	19	Severe migraine twelfth to sixteenth year	Convulsions—Father twenty third to thirty fifth year
									Petit Mal at 15	Migraine—Mother
									Asthma Hives	Alcoholism—Father
*39	36	M	5	Nor	Br	No	15	3	None	Hives—Sister
40	37	M	3	Nor	Br	No	14	34	First convulsion at 18 years	Migraine—Mother
									None then until pregnant	Asthma—Father
41	22	S	7	Nor	Br	No	13	18	Eczema and hives	Asthma—Father
42	17	S	1	In	Br	No	12	14	Migraine and eczema	Hay fever—Brother
									Sensitive skin	Hives—Sister
43	27	M	3	Pre	Bot	Yes	13	14	Convulsions with enteritis first year (Scarlet fever at 8)	Migraine—Mother
									Petit Mal after 8	Migraine—Mother and sister
										Epilepsy—Paternal cousin
										Migraine—Mother and maternal grandmother

* Wassermann negative

TABLE II
TABLE SUMMARIZING THE HISTORY OF 57 ADULT MALE EPILEPTICS

CASE NO	AGE	MARRIED OR SINGLE	ORDER OF BIRTH	BIRTH NORMAL OR INSTRUMENTAL	BREAST OR BOTTLE FED	CONVULSIONS IN INFANCY	AGE WHEN DIAGNOSED FOR EPILEPSY	IN PATIENT	ALLERGIC MANIFESTATIONS	IN ANCESTORS AND FAMILY
1	41	M	3	Nor	Br	No	39	None obtainable		<i>Hives</i> and <i>urticaria</i> —Father
2	53	M	3	Nor	Bot	Yes	49	Convulsions with <i>digestive</i> 'upsets' first to ninth year (began to smoke), none then until 19		<i>Hives</i> —Mother, maternal aunt and cousin <i>Convulsions</i> in 2 paternal uncles when young <i>Convulsions</i> in 2 sisters and 1 brother when young <i>Convulsions</i> in son of patient at twentieth year
3	31	S	1	Nor	Br	No	12	None obtainable		<i>Migraine</i> —Father and maternal grandmother <i>Asthma</i> —Maternal grandfather and aunt
4	20	S	3	Nor	Bot	Yes	14	<i>Digestive disturbances</i> in infancy and childhood		<i>Epilepsy</i> —Paternal cousin
5	34	M	1	Nor	Bot	Yes	13	<i>Periodic vomiting attacks</i> with <i>headaches</i> through childhood		<i>Asthma</i> —Mother from childhood
6	24	S	4	Nor	Br	Yes	19	<i>Digestive disturbances</i> and <i>diarrhea</i> in childhood <i>Convulsions</i> thru summer		<i>Migraine</i> —Mother 2 mat aunts and 1 uncle <i>Eczema</i> —Father
7	17	S	1	Nor	Br	No	14	(Scarlet fever at 7) <i>Menigitis</i> (?) at 13 with convulsion		<i>Hay fever</i> —Brother <i>Convulsions</i> —Sister when teething <i>Migraine</i> —Mother and mat grandmother and aunt <i>Epilepsy</i> —Maternal cousin
8	15	S	4	In	Bot	Yes	11	<i>Digestive disturbances</i> through infancy with first convulsion at 2 years Second convulsion at 5 eating watermelon		<i>Asthma</i> —Paternal grandfather <i>Bilious Attacks</i> —Father
*9	31	M	5	Nor	Br	No	28	First convulsion after eating "hot dogs," and convulsions since, always with digestive "upsets,"		<i>Hay fever</i> —Migraine and <i>Eczema</i> —Father <i>Asthma</i> —Paternal uncle <i>Migraine</i> —with vomiting—Mother and mat aunt <i>Hay fever</i> —Older brother <i>Asthma</i> —Mother for years <i>Migraine</i> —Sister
*10	42	M	4	Nor	Br	No	27	None obtainable		<i>Asthma</i> —Mother since childhood
*11	26	S	2	Nor	Bot	No	19	First convulsion at 19 after eating hot poppers		<i>Migraine</i> —Mother maternal grandmother and aunt <i>Convulsions</i> —Brother in infancy

*Waassermann negative.

TABLE II—Cont'd

CASE NO	AGE	MARRIED OR SINGLE	ORDER OF BIRTH	BIRTH NORMAL	OR INSTRUMENTAL	BREAST OR BOTTLE FED	CONVULSIONS IN INFANCY	AGE EPILEPSY DIAGNOSED	IN PATIENT	ALLERGIC MANIFESTATIONS	IN ANCESTORS AND FAMILY
12	23	S	1	Nor	Nor	Bot	No	19	Much digestive trouble in childhood <i>Asthma</i> for 6 months during twenty first year and convulsions absent	<i>Hives</i> —Father thirteenth to fortieth year, also <i>Migraine</i> <i>Convulsions</i> —Paternal uncle nineteenth to twenty fifth year <i>Hay Fever and Asthma</i> —Sister <i>Migraine</i> —Mother <i>Convulsions</i> —Sister when teething	
13	24	S	1	Nor	Nor	Br	Yes	14	<i>Convulsions</i> accompanied all febrile disturb- ances from first to sixth year, none then until 14	None obtainable	
*14	31	M	16 (Twin)	Nor	Nor	Bot	Yes	29	Much digestive disturbance with convulsions when child (Scarlet fever at 10)		
*15	39	S	2	Nor	Nor	Br	No	33	<i>Migraine</i> with vomiting when boy and young man		
16	21	S	2	Nor	Nor	Br	No	19	<i>Migraine</i> since boyhood	<i>Hives</i> and <i>Severe Migraine</i> —Mother	
*17	17	S	2	Nor	Nor	Bot	No	14	<i>Digestive disturbances</i> frequent in infancy (Scarlet fever at 3) <i>Petit Mal</i> 10 to 14	<i>Migraine</i> —Father <i>Asthma</i> and <i>Cyclic Diarrhea</i> —Mother <i>Migraine</i> —Father and maternal aunt <i>Asthma</i> —Paternal grandmother <i>Hives</i> —Sister	
18	37	M	3	In	In	Bot	Yes	22	<i>Digestive disturbances</i> in infancy "Cyr sickness" as boy	<i>Hives</i> and "Bilious Spells"—Mother <i>Eczema</i> —Two maternal aunts and patient's son <i>Migraine</i> —Father <i>Eczema</i> —Two maternal aunts and patient's son <i>Migraine</i> with vomiting—Mother <i>Hay Fever</i> —Maternal aunt	
*19	30	S	1	Nor	Nor	Bot	No	15	<i>Digestive disturbance</i> marked in infancy <i>Convulsion</i> at 7		
*20	15	S	2	In	In	Bot	Yes	10	<i>Petit Mal</i> to 15, <i>Grand Mal</i> since <i>Digestive disturbances</i> with vomiting spells and <i>Periodic diarrhea</i> until fourth year <i>Petit Mal</i> followed <i>convulsion</i> at 15 months and <i>Grand Mal</i> at 10	<i>Hives</i> , "Bilious Attacks," <i>Con</i> when teething— Father <i>Hay Fever</i> —Mother, maternal grandmother and ma- ternal uncle <i>Migraine</i> and <i>Hives</i> —Sister <i>Migraine</i> —Mother	
*21	51	M	2	Nor	Nor	Br	No	48	<i>Hives</i> <i>Hay Fever</i> since a boy <i>Ivett Bed</i> until 12	<i>Asthma</i> —Maternal grandmother <i>Hay Fever</i> —Paternal grandmother <i>Epilepsy</i> —Paternal aunt and cousin <i>Hives</i> —Sister	
*22	19	S	3	Nor	Nor	Bot	Yes	17	<i>Periodic Vomiting</i> until 7 with convulsion		

*Wassermann negative.

TABLE II—CONT'D

*23	31	S	2	In	Br	Yes	17	Convulsion second day and again during second year with much indigestion	<i>Hives</i> —Mother <i>Asthma</i> —Maternal grandfather and uncle
24	26	M	6	Nor	Br	No	16	None then until 17 after eating banana Convulsions during convalescence from typhoid at 9 attributed to eating can of Nabisco's	Convulsions—Paternal uncle <i>Asthma</i> —Father <i>Migraine</i> —Older sister Convulsions—Brother in infancy <i>Hives</i> —Younger sister <i>Hay Fever</i> —Mother for 30 years <i>Migraine</i> with vomiting—Maternal grandmother
25	21	S	1	Nor	Bot.	Yes	14	<i>Digestive "upsets"</i> with a convulsion when teething and convulsion at two and one half after eating pork <i>Asthma</i> seizures from first to seventh year <i>Hives</i> from strawberries	None obtainable <i>Asthma</i> —Maternal grandfather <i>Migraine</i> —Maternal grandmother <i>Migraine</i> —Mother <i>Asthma</i> —Maternal aunt
*26	24	S	3	Nor	Br	No	16	First convulsion after "Flu" at 16	None obtainable
*27	27	S	1	Nor	Bot	No	20	<i>Gastric Disturbances</i> marked in infancy <i>Pet Red</i> until 10	<i>Asthma</i> —Paternal grandmother
28	25	S	4	Nor	Bot	Yes	12	First convulsion at 12— <i>Petit Mal</i> after 20 Enteritis with vomiting spells when infant and convulsions until 2	<i>Migraine</i> —Mother <i>Asthma</i> —Maternal aunt
*29	46	M	3	Nor	Br	No	28	<i>Ec ems</i> and <i>Hives</i> when boy <i>Amoebic dysentery</i> in Cuba at 19 Typhoid in China at 23	None obtainable
30	16	S	2	In	Bot	No	14	<i>Hay Fever</i> each fall for 6 years	<i>Ec ems</i> —Mother
31	15	S	4	Nor	Br	No	11	None	<i>Asthma</i> —Maternal uncle
32	17	S	3	In	Bot	Yes	16	<i>Digestive Disturbances</i> and Enteritis with Convulsions at 2 years	<i>Asthma</i> —Paternal grandfather Convulsions—Paternal grandmother and 2 paternal aunts
*33	32	S	3	Nor	Br	No	18	<i>Pet Red</i> until 14 (Scarlet fever at 12)	Convulsions when teething—Sister
34	21	S	5	In	Bot	Yes	18	Diarrhea second summer and Convulsion <i>Nephritis</i> at 3 with Convulsions <i>Hives</i> until 14	<i>Asthma</i> —Paternal grandfather and maternal grandmother <i>Migraine</i> —Paternal grandmother Convulsion—Paternal uncle
35	15	S	4	Nor	Bot	Yes	14	<i>Digestive Disturbances</i> second summer with Convulsions	<i>Ec ems</i> —Sister <i>Migraine</i> —Father and mother until menopause
36	23	S	2	Nor	Br	No	6	Started to wet bed at 10 Convulsions after eating veal (Scarlet at 6) Convulsions started 2 months later and have continued	<i>Hives</i> —Mother since menopause Convulsions—Paternal grandfather when young man <i>Hay Fever</i> —Father
*37	23	S	1	Nor	Br	No	21	Menstruation at 8 with convulsion	<i>Migraine</i> —Mother until menopause <i>Asthma</i> —Grandmother <i>Migraine</i> —Mother
*38	45	M	5	Nor	Br	No	40	<i>Pet Red</i> when boy <i>Petit Mal</i> at high school	

Wassermann negative

TABLE II.—Cont'd

CASE NO	AGE	MARRIED OR SINGLE	ORDER OF BIRTH	BIRTH, NORMAL OR INSTRUMENTAL	BREAST OR BOTTLE FED	CONVULSIONS IN INFANCY	AGE EPILEPSY DIAGNOSED	IN PATIENT	ALLERGIC MANIFESTATIONS	IN ANCESTORS AND FAMILY
*39	48	M	2	Nor	Br	No	46	(Scarlet Fever at 4) Petit Mal at 38 Periodic Headaches last five years		Migraine—Mother and maternal aunt Hives and Eczema—Sister Asthma—Maternal grandmother Migraine—Mother Migraine—Mother Asthma—Father Epilepsy—Paternal grandfather
40	16	S	1	Nor	Bot	Yes	15			
41	15	S	4	Nor	Bot	Yes	20	Petit Mal at 10 years		
42	25	M	2	In	Br	No	16	Severe Migraine as school boy		
*43	26	S	3	Nor	Br	No	19	Nono		
*44	19	S	1	Nor	Bot	No	17	(Scarlet Fever at 6 years)		
45	24	S	2	In	Br	No	15	Periodic Diarrhea "Bilious Spells" when child		
*46	15	S	3	Nor	Bot	Yes	14	Nono		
47	35	M	1	Nor	Br	Yes	31	Food hypersensitivity since childhood		
*48	27	S	4	Nor	Br	No	19	Digestive upsets preceded convulsions		
49	29	S	1	Nor	Br	No	4	Convulsions after a tumbler of whiskey at 4 years		
50	28	S	8	Nor	Br	No	19	Petit Mal at 11 years		
51	19	S	3	Nor	Br	Yes	14	Scarlet Fever at 6 years		
52	22	S	1	In	Br	No	19	Migraine since twelfth year		
53	17	S	4	Nor	Br	No	3	Chorea at 7 and 9 years—Petit Mal at 8 Eczema, two severe attacks		
54	20	S	1	In	Br	No	15	Rickets (Scarlet Fever at 12)		
55	15	S	8	Nor	Br	No	13	First attack in sun, picking berries		
56	35	S	1	Nor	Br	Yes	14	First Convulsion at 2 years after eating eggs none then until 12		
*57	38	M	1	In	Br	Yes	34	Migraine since childhood Eczema		

*Wassermann negative

TABLE III

SUMMARIZED DEDUCTIONS OF TABLES I AND II

57 Males 15 married	43 Females, 14 married
Age Epilepsy Diagnosed grouped in decades	
First to ninth year inclusive	5
Tenth to nineteenth year inclusive	68
Twentieth to twenty ninth year inclusive	15
Thirtieth to thirty ninth year inclusive	8
Fortieth to forty ninth year inclusive	4
	<u>100</u>

25 First Born	{	16 Breast Fed	2 Conv in Infancy	31 2 per cent
		9 Bottle Fed	4 Conv in Infancy	44 4 per cent
		10 Breast Fed	3 Conv in Infancy	30 per cent
20 Instrumentally Delivered	{	10 Bottle Fed	8 Conv in Infancy	80 per cent

Bottle Feeding (protein sensitivity to milk or food foreign to the mother) may be a more potent factor in the development of infant convulsions than a difficult or instrumental birth

In 62 patients Breast Fed	8 had Convulsions in Infancy	12 9 per cent
In 38 patients Bottle Fed	24 had Convulsions in Infancy	63 1 per cent

Gastrointestinal hypersensitivity to cow's milk or other substitute for mother's milk appears to be a decided factor in the development of infant convulsions in the atopic child

TABLE IV

HEREDITARY DEDUCTIONS FROM TABLES I AND II

SUMMARY OF 100 (43 FEMALE AND 57 MALE) ADULT CASES OF EPILEPSY SHOWING THE INCIDENCE OF ALLERGIC MANIFESTATIONS IN THE PATIENT IN THE BROTHERS AND SISTERS, AND IN THE ANCESTORS (PARENTS GRANDPARENTS UNCLES AND AUNTS)

ALLERGIC MANIFESTATIONS	PATIENT	MATERNAL					PATERNAL					TOTAL NUMBER ANCESTORS SHOWING ALLERGIC MANIFESTATIONS
		BROTHERS	SISTERS	MOTHER	GRANDMOTHER	GRANDFATHER	FATHER	GRANDMOTHER	GRANDFATHER	AUNTS	UNCLES	
Asthma	3	1	1	8	8	4	3	5	8	2	5	44
Hay Fever	3	4	1	2	1	4	3	2	3	1	5	12
Hives (Pruritis)	18	2	9	10			4		4			17
Eczema	11	1	5	2			3	1				8
Migraine	10		7	38	9	1	15	3		2		77
Convulsions		5	10	1	1	2	3	1	3	4	6	24*
Totals	54	13	33	61	19	7	36	8	8	6	7	182
		46		117			65					

83 per cent of the 100 cases showed a history of allergy in the ancestors

12 per cent of the 100 cases showed no history of allergy in the ancestors, but in 5 of these, convulsions occurred in the ancestors

*8 in infancy only

marked irritability of the central nervous system with violent convulsions in guinea pigs, and it would seem equally possible that in certain predisposed human beings outbreaks of allergy might produce epileptic attacks. Nerve strain increases hypersensitiveness in the allergic individual and we know that certain foods ingested by an allergic while under nervous strain produce sensitization manifestations which are not induced by eating the same food in the absence of nervous influences. It is probable, however as Van Leeuwen¹ points out, that a primary factor exists in the epileptic patient which predisposes the brain centers, and that the allergic reaction may act as a secondary stimulus.

The production of convulsive seizures by the transfusion of blood from an epileptic to a nonepileptic individual, as reported by Boston and Henry,² shows the possibility of transference of epileptic allergic sensitization in the human. The convulsions occurring in animals following the injection of blood from a human suffering with epilepsy, as reported by Cuneo,³ Antheaume and Trepsat,⁴ and others, proves that human epileptic sensitization can be transferred to animals.

LABORATORY EVIDENCE

Food sensitization tests were made on 19 of the patients in this series and in 6 of them were repeated a number of times. The results on the whole were negative and I have not tabulated them for this report. My experience with food skin tests in epilepsy has been disappointing.

Food history, i.e., tabulating each day all articles of food taken, has in a number of cases proved of decided value and it has been possible to eliminate from the diet of some patients certain foods which proved to be factors in precipitating convulsions.

The ketogenic or high fat content diet may owe its value, I believe, from the cases in which I have used it, more to the accidental elimination of the specific protein to which an epileptic patient is sensitized, than to the production of acidosis or ketone bodies in the system.

The basis of allergic reactions has been shown by Hanslik⁵ and others to rest fundamentally on disturbances in the chemical and physical mechanisms of the blood and tissues. A study of the blood, therefore, is of much practical value in allergic epilepsy as well as in other sensitization diseases.

In a previous paper⁵ on "The Blood in Sensitization Diseases" my experience has been detailed and I will only mention this time several of the conclusions.

1 *Chemical blood findings* that have been found uniformly in epilepsy and other sensitization diseases include an increase of uric acid, a lowered alkali reserve and a calcium deficiency.

2 *Physical blood characteristics* in allergic conditions including epilepsy are

(a) An *eosinophilia* as the most definite and constant blood finding of allergic phenomena if search is made by frequently repeated counts.

(b) A primary *leucopenia* followed by a *polymorphonuclear leucocytosis* when the symptoms arise from inhaled or ingested allergens, and a lymphocytosis at times when the symptoms arise from chronic foci of infection.

Table V illustrates the effect of venom protein desensitization on the red and white blood count and the modification of the blood count by a convulsive seizure.

Table VI illustrates venom protein desensitization and the effect of a convulsion on the differential leucocyte count. Attention is called to the increase of the polys following a convulsion and the increase of eosinophils following the injections.

TABLE V

DAY OF MONTH AND HOUR	RELATION OF BLOOD COUNT TO TIME OF INJECTION OR CONVULSION	LEUCOCYTES	ERYTHROCYTES
12 noon	Just before injection	8,000	4,400,000
10 P M	10 hr after injection	7,600	3,600,000
13 10 P M	34 hr after injection	7,800	4,700,000
10 8 A M	4 hr before injection	7,200	3,700,000
10 10 P M	10 hr after injection	7,000	3,100,000
20 8 A M	24 hr after injection	8,200	4,000,000
6 P M	Convulsion		
10 P M	4 hr after convulsion	18 000	3,100,000
21 8 A M	14 hr after convulsion	20,000	2,750,000
7 P M	2 hr after convulsion	10 200	3,700,000
22 8 A M	38 hr after convulsion	7,000	3,000,000
24 7 P M	Just before injection	7,600	3,200,000
25 9 A M	14 hr after injection	6,800	4 200,000
7 P M	24 hr after injection	6,400	3,750 000
26 8 A M	36 hr after injection	7,200	3 950 000

TABLE VI

DAY OF MONTH	RELATION OF COUNT TO TIME OF INJECTION AND CONVULSION	POLI	S L	L L	EOSIN	BASE	TRANS
2	Before injection	61	20	4	3	-	6
4	42 hours after injection	59	27	2	8	1	4
9	Before injection and 5 hours after convulsion	88	9	1	-	-	2
11	45 hours after injection	61	18	3	8	1	9
16	Before injection	70	14	4	1	1	10
18	48 hours after injection	58	23	5	6	-	8
23	Before injection	65	24	2	2	-	7
25	42 hours after injection	55	22	5	9	1	8
29	Before injection	71	20	1	3	-	5
31	45 hours after injection	57	29	2	6	-	6

HEREDITARY EVIDENCE

The allergic or atopic hereditary characteristics are a striking feature in this series of epileptic individuals as summarized in Table IV. While in the ancestors of 24 of the 100 cases there was a history of convulsions, we find on close analysis that only one mother and two fathers had convulsions which could be diagnosed as epilepsy, i.e. in 3 per cent of these patients a parent had epilepsy. In a third father there was a history of convulsions which did not persist after infancy. In 7 of the grandparents convulsions occurred 3 on the maternal and 4 on the paternal side. In the uncles and aunts (collateral relatives) there were 13 instances of convulsions.

Turning now to other allergic manifestations we find that there were 77 instances of *migraine* among the ancestors of these 100 epileptic patients. Fifty seven of these occurred on the maternal side and 20 on the paternal side, a proportion of nearly 3 to 1. *Migraine* was found in 38 of the mothers and 15 of the fathers, i.e. two and one half times more frequent in the mothers than in the fathers.

Asthma occurred in 44 of the ancestors, 28 instances on the maternal side and 16 on the paternal side. *Urticaria* was found in 17 of the ancestors, 13 on the maternal side and 4 on the paternal side. *Hay fever* 12 times 8 on maternal and 4 on paternal side. *Eczema* 8 times, 4 maternal and 4 paternal.

To summarize, there was a total of 182 instances of allergy in the ancestors (the parents, grandparents, uncles and aunts) of these 100 adult epileptic individuals. One hundred and seventeen occurred on the maternal, 65 on the paternal side of the families. In 88 per cent of the 100 patients there was a history of allergy in one or more forms among the ancestors. In 12 per cent of the 100 cases there was no history of allergy in the ancestors, but in 5 of these patients convulsions were present in the ancestral history.

In the brothers and sisters of these 100 epileptic patients there were 46 clinical instances of allergy, 33 among the sisters and 13 among the brothers. Ten sisters and 5 brothers had convulsions, 9 sisters and 2 brothers hives, 7 sisters and no brothers migraine, 5 sisters and one brother eczema, 4 brothers and 1 sister hay fever, and 1 brother and 1 sister asthma. Here again there were more instances of allergy on the female side in a proportion of more than 2 to 1.

ENDOCRINES, ALLERGY AND EPILEPSY

Out of 43 adult females in the present series, 7 had their first convulsion before menstruation was established, in 4 the first convulsion occurred during the first menstrual period and 32 had their first convulsion after menstruation was established.

Clinically menstruation is often regarded as a factor in the etiology of epilepsy in the female, and probably accounts for some diagnoses of endocrine epilepsy. In some patients the proximity or appearance of the menstrual cycle very frequently is accompanied by an epileptic seizure. In other patients there seems to be no relation between menstruation and epileptic symptoms.

Charles Hajos (*Endocrinology*, x, No 6, p 560) in discussing the relationship between clinical symptoms in allergic states and the activity of the internal secretions reports that no difference in appearance or shock symptoms could be shown experimentally in sensitized guinea pigs when injected intraperitoneally with either ovarian extract or horse serum. He concludes that "Change in the function of the female sex glands may either increase or decrease clinical anaphylactic sensitiveness." These experiments probably help to explain the clinical allergic variations found in the female epileptic in connection with menstruation.

CLINICAL EVIDENCE OF ALLERGY IN EPILEPSY

The first signs of allergy often show themselves early in infancy. When for one reason or another cow's milk or other substitute food replaces mother's milk there may appear, as we have all experienced, gastrointestinal upsets, at times with periodic vomiting, cyclic diarrhea, various digestive disturbances, or such evidences of allergy as eczema, hives, etc. It is under these conditions that infant spasms or convulsions are apt to appear. In the 100 cases summarized in Tables I and II there was a history of digestive disturbances with convulsions in infancy in 32 of the patients. Of these thirty-two, twenty-four (or 75 per cent) were bottle-fed and eight (or 25 per cent) were breast-fed infants. The so-called "reflex spasms" in infants especially those attributed to teething will often be found on close analysis, I believe, to be

evidence of protein hypersensitivity which occurs coincidentally with the change in the form of diet the child ingests when teething

Convulsions associated with infectious diseases seem to be much more likely to occur in children with a hereditary allergic history and in those children who have clinical allergic manifestations than in children whose personal and family histories are negative to allergic phenomena. Sixteen in this series of 100 had scarlet fever before epilepsy started. The incidence of other infectious diseases was not tabulated.

It is a rather common observation among epileptic patients, as it is among patients with other forms of allergy, to find more than one allergic clinical manifestation. In fifty-four of these 100 patients with recurring periodic convulsions there occurred the following other allergic conditions: Nineteen had migraine (independent of the headaches associated with convulsive seizures), eighteen were subject to hives, eleven had eczema, three asthma and three hay fever. Then too there was a history of bed wetting before any convulsion had occurred in a number of these patients in whom hypersensitivity of the unstriated muscle fibers of the bladder must be considered.

At times clinical evidence of the allergic nature of epilepsy is striking. By careful inquiry and with cooperative observation on the part of the patient, it is possible in some cases of epilepsy to determine that a certain article of diet is acting as the offending allergen. A few case reports will illustrate some of the above observations.

CASE 1—A 15 year old boy who was a bottle fed infant had digestive disturbances and convulsions his second summer. At 10 he started to wet the bed. At 14 he began to have recurring convulsions and epilepsy was diagnosed. When he came under my care he was having, with but few exceptions, a convulsion Sunday or Monday of each week. Food skin tests were negative. Food history, with daily tabulation of all foods ingested for seven weeks showed that six out of the seven weekends on Saturday or Sunday some form of real was eaten. Over the seventh week end no real was taken and that was the only week end no convulsion occurred. Since then, now 22 months ago, no real has been eaten and no convulsions have occurred.

This boy's father suffered with severe migraine until he was relieved by six months nonspecific desensitization treatment with crotalin. His mother too suffered with migraine until the menopause, and since then, for the past 5 years has from time to time developed hives. His paternal grandfather had convulsions when a young man.

CASE 2—February 1926 Dr. I. R. Strawbridge, gastroenterologist, referred for study and investigation a 9 year old girl with the following history. She was the youngest of seven children, a full term, normally born infant. Breast fed for six months then among other foods was given a soft boiled egg, which was followed by severe gastrointestinal symptoms and a convulsion. Subsequent convulsions occurred at the sixth month, third, fifth and eighth years. All were believed by family to be associated with attempts at eating egg in some form. Between the convulsive seizures there occurred every few weeks periodic attacks of "abdominal cramps" at times with vomiting and accompanied by "dazed states" with a stare, automatic picking at fingers and clothes and followed by slight mental confusion. Two weeks before I first saw the patient she developed "status" when one convulsion rapidly followed another for one and a half hours. An x-ray study of the stomach and intestinal tract revealed no pathology or abnormality.

The allergic disposition running through the maternal side of this child's family is significant. Her mother suffered with severe migraine and periodic vomiting from childhood. Maternal grandfather had "spasms" when a boy. A maternal aunt had migraine, and

another maternal aunt suffers with hives. This aunt had a child die at 18 months of age, who was ill only 36 hours and death was attributed by a very competent pediatricist to anaphylaxis following ingestion of a soft boiled egg. An older sister of this patient has had mild seasonal hay fever, an older brother had chorea at 8 years and another brother died with convulsions when 2 years old.

It is of interest that following desensitization with venom protein (crotalin) solution at weekly intervals, in doses ranging from 1/600th to 1/300th grain, no convulsions or abdominal symptoms occurred from February 16 to June 4. The interval between injections was lengthened to two weeks, and June 9 there were mild abdominal symptoms. June 11, 1/300 grain of crotalin solution was given intramuscularly (without producing an eosinophilia) and on June 19 an apparent series of convulsions began. After the second seizure in an hour, 20 min of adrenalin 1:1000 solution was given hypodermically. After a third convulsion 2 hours later a second hypodermic of adrenalin was used and the threatened series ended. At this time no definite history of egg ingestion could be obtained but many cherry stones were found in her stool after an enema. This patient developed an eosinophilia ranging from 7 to 13 per cent following weekly injections of 1/400 to 1/300 grain doses of crotalin over a period of four months. When the interval between injections was lengthened to two weeks an increase of eosinophils did not occur and a series of convulsions came on. The strength dose of crotalin was then increased gradually to 1/150 grain and an eosinophilia ranging from 5 to 13 per cent in the differential count has followed injections at two weeks' interval. There have been no convulsions and but three evidences of abdominal symptoms, which were very mild, in the past eleven months.

CASE 3—January 1, 1917 Dr. Andrew Callahan referred a 37 year old married man to be treated for convulsions. This patient was born in Austria. He never knew his father, and his mother died when he was a child. He served in the Austrian army until his twenty fifth year, when he was given a sick leave owing to nocturnal convulsions. He came to America and eight months later had a convulsion, another in six weeks, and from the twenty seventh to the thirty seventh year had seizures at from three to six week intervals. He thought the attacks frequently followed the eating of pork.

Pork was excluded from his diet and crotalin injections were given at weekly intervals in doses ranging from 1/200 to 1/75 grain. His eosinophil increase ranged from 5 to 14 per cent for three months and after that, with but slight local recurrences their percentage kept within the normal range (4 per cent or under). The patient was entirely free from convulsions from December 29, 1916, to December 26, 1917 and had eaten no pork. Christmas Day, 1917 he ate roast pig for dinner and at 1 A.M. that night, December 26, had a convulsion. Injections were continued at lengthened intervals until the end of the second year with freedom from attacks and since then he has reported at the office every second or third month. No injections, no pork and no convulsions. On October 24, 1922 (5 years less 2 months after his last seizure) his wife phoned me he had had a convulsion. Inquiry showed that he had unknowingly eaten pork bologna. After another year of desensitization, treatment was stopped again. I saw the patient two weeks ago and no convulsions have occurred for a second interval of now nearly five years. He reports, however, that during the past year he has had four or five very severe attacks of migraine with vomiting.

CASE 4—March 14, 1926, Dr. Joseph O'Neil referred a 21 year old, single female stenographer who had had two convulsive seizures within three weeks. Her history revealed that she was a twin of a third pregnancy. She was a full term baby and had had a normal birth. She was breast fed for three months and then given modified cow's milk by bottle. With the change from mother's milk, digestive disturbances began, vomiting and diarrhea of a rather cyclic type and eczema developed (hypersensitivity to change of protein). Teeth were erupted at usual age and no convulsions occurred in childhood, although she had measles, mumps and scarlet fever before the twelfth year. Menstruation was established at thirteen, always regular, but periodic headaches with vomiting, which had been present since childhood, began to recur rather regularly at her periods. The eczema persisted from childhood until the eighteenth year when it gradually became better and finally disappeared. At this time patient began to notice momentary lapses of consciousness (petit mal) with "dizzy

spells" which would recur at irregular intervals varying from a few days to a week or occasionally a month's interval. In her twenty first year the first convulsions appeared.

This patient's father has had eczema for years. Paternal grandfather also long suffered with eczema. Her mother "gets hives" and maternal grandmother was subject to periodic headaches with vomiting. A maternal aunt is said to have had a four year old son die of hives. Patient's older brother had hives when a boy and now is subject to migraine.

Venom protein desensitization was given at weekly intervals for four months with an eosinophilia ranging from 6 to 10 per cent, and then the injections lengthened to two week intervals. All petit mal seizures stopped and there were no convulsions until January 17, 1927 (a ten month interval) when two convulsions occurred at 6 and 10 P.M. The patient attributed these to eating half a pound of jelly eggs. Since then, now four months, the eotablin injections have been continued at two week intervals with the strength dose increased from 1/200 to 1/100 grain and no further petit mal or convulsions have occurred.

Rarely in my experience with epilepsy does the offending allergen gain entrance to the system by inhalation. The only instance in my study of over 700 epileptic patients in private practice when an inhaled allergen has been definitely shown to be a factor is the following patient.

CASE 5—A 38 year old married man engine inspector by occupation, had his first convulsion at 34 years of age. There was however a history of recurring series of momentary lapses, petit mal, for several years before the convulsions. He had 2 negative blood and one negative spinal fluid Wassermann tests. The year before I saw the patient, while taking luminal, he had 21 convulsive seizures with series of petit mal between the major attacks. Eotablin injections, with an increase of eosinophils as high as 18 per cent, were given intramuscularly at weekly intervals over a period of six months and only three convulsions occurred, but the petit mal continued in series of 8 to 10 in 24 hours, about every two weeks.

The patient was then advised to give up cigarettes. He had been smoking and inhaling 20 to 40 daily. He complied. By the end of two months, with the continued weekly injections of eotablin, no convulsions occurred, but the petit mal continued as frequently as before and the patient told me he thought he would go back to cigarettes again as he was getting very tired of chewing. I then learned for the first time that he had started chewing when he stopped smoking. Tobacco in all forms was then stopped and the eotablin injections have been continued at three week intervals. Nearly two years have now passed and no convulsions or petit mal seizures have occurred. The patient has gained 38 pounds in weight and all apprehensive symptoms have disappeared. His skin tests to tobacco, however, continue to give a fairly marked reaction.

This patient had convulsions in infancy. He was instrumentally born but breast fed. He had suffered with severe migraine since childhood and for several years had a dry scaly eruption on both forearms. The headaches and skin condition disappeared with nonspecific desensitization before tobacco was stopped. His mother and a maternal aunt had migraine and there was also a history of 'strawberry rash' in the mother. A maternal uncle suffers with asthma.

THERAPEUTIC EVIDENCE

In the recent report of Hajos referred to above he concludes "that experimental and clinical anaphylactic sensitiveness are decreased by adrenalin, by extract of the parathyroids and the posterior lobe of the pituitary." These conclusions correspond with the rather uniform experience of physicians who have used the endocrine extracts as antiallergic agents. The use of adrenalin, atropine and calcium for the relief of attacks of asthma, hay fever and urticaria are established therapeutic measures. Their value in anaphylactic and anaphylactoid states is also now well recognized.

When we come to allergic epilepsy, i.e., those cases in whom there can be

obtained a history of familial hereditary hypersensitiveness and associated clinical and laboratory evidence of allergic manifestations in the patient, the treatment may be divided into treatment of the attacks and treatment during the interval between seizures. The hypodermic injection of adrenalin—(15 to 20 minims) at the time of seizures of convulsions and as I recently experienced in two cases of "status epilepticus," has been of much aid in my hands. The routine daily use of adrenalin in petit mal series and as a preventive measure for grand mal, however, has not proved of much benefit. I have had no experience with ephedrin.

The treatment during the interval between epileptic seizures has been, in the past, largely by administration of bromide and luminal. In recent years with evidence pointing to the anaphylactic nature of some epileptic attacks various so-called specific and nonspecific agents have been employed as desensitizing and antiallergic measures.

NONSPECIFIC DESENSITIZATION

The exact mechanism of antiallergic treatment is not definitely known. Specific desensitization is based largely on the fact that the injection of small amounts of an allergen can reduce the hypersensitiveness of an individual to that allergen and thus prevent or modify the clinical symptoms. In many allergic individuals, however, the specific allergen or causator agent cannot be determined and therefore specific desensitization is not applicable. This is especially true in the present state of our knowledge, in those epileptic patients who have an allergic heredity and in whom allergic symptoms (such as asthma, hay fever, urticaria, eczema, migraine, etc.) are present at the same time or appear independent of the convulsive symptoms.

It has been learned, however, from clinical experience and confirmed by the experiments of Longcope,⁷ Dale,⁸ and others that hypersensitiveness to one allergen may be reduced by the injection of small quantities of another allergen provided the patient or animal, is found to be hypersensitive, i.e., reacts locally and systemically to the agent or allergen injected. This fact may help to explain so called "infection immunity," that is the relief of asthmatic paroxysms, migraine, seizures, and epileptic attacks which at times takes place in allergic individuals who contract an infectious disease, or during pregnancy and at times following operations.

These observations and clinical experiences have led to the application of nonspecific desensitization therapy, by the use of various agents. First, in those allergic patients in whom a definite causative agent cannot be determined, second, in those cases in whom the cause is known, but in whom specific treatment gives but partial or no relief, and third, at times also in that smaller percentage of allergic patients who react so severely to even the minutest doses of the causative agent, that the danger of inducing heightened sensitiveness prohibits the use of the specific allergen as a therapeutic measure.

NONSPECIFIC AGENTS EMPLOYED

A large variety of substances including such proteins as peptone, proteoses, milk, various serums, also colloidal metals, a number of drugs (sulphur,

turpentine, iodides) and distilled water are being used to produce nonspecific reactions for desensitizing and therapeutic purposes. The chief difference clinically in the various agents employed is probably largely in the intensity of the phenomena induced.

My experience with nonspecific protein therapy has been very largely confined to the use of venom protein (crotoalin) solution. I will not review the relative values of the various agents used or the practical advantages in the use of crotoalin solution which I have covered in detail in previous papers.⁹

Not all cases of epilepsy are found to be hypersensitive to crotoalin, but those who can be sensitized by small intramuscular injections have been found to indicate their reactivity by a mild local reaction at the site of the injection and by an increase in the percentage of eosinophil cells in the differential blood count in from 12 to 48 hours after an injection.

PROGNOSTIC VALUE OF EOSINOPHILIA

I would emphasize again the need of a scientific method for regulating the strength of dose and for determining the frequency with which both specific and nonspecific agents should be administered. Beside the advantages offered by the mild local reaction and the absence of any severe systemic symptoms (chill, fever, shock, etc.) following intramuscular injections of crotoalin solution, it is an agent that does not lower alkalinity as do many other nonspecific substances and I have found the degree of eosinophilia produced in the differential blood counts to be of prognostic value as well as a very satisfactory guide to dosage.

From my experience in giving more than 18 000 injections of venom protein (crotoalin) solution to over 700 epileptic patients in private practice during the last nineteen years it has been found that in those cases in whom an increase in the percentage of eosinophils does not occur in from four to six weeks following weekly injections clinical improvement of the symptoms will be doubtful. In other words an increase of eosinophil cells following venom protein injections may be used as an indication of the *reactive power of the individual*.

METHOD FOR REGULATING DOSE

In those patients who can be sensitized to crotoalin solution as a rule the highest percentage of eosinophils following the range of doses ($\frac{1}{400}$ to $\frac{1}{50}$ grain) which I have used occurs within forty-eight hours. In from five to eight days after an injection the eosinophil cells will as a rule, have dropped to 4 per cent or under and the patient may be given another injection. It has been my practice not to repeat an injection if the percentage of eosinophil cells by the fifth day has not dropped to at least 4 per cent (Howell's high level of the normal range). Moreover, it has not seemed wise to increase the strength of dose as long as any given strength is producing an 8 to 10 per cent eosinophilia on the second or third day after an injection.

To summarize—Clinical and laboratory experience indicate that in an epileptic patient, with an allergic family and personal history, whom it is possible to sensitize to small doses of venom protein (crotoalin) solution as

obtained a history of familial hereditary hypersensitiveness and associated clinical and laboratory evidence of allergic manifestations in the patient, the treatment may be divided into treatment of the attacks and treatment during the interval between seizures. The hypodermic injection of adrenalin—(15 to 20 minims) at the time of seizures of convulsions and as I recently experienced in two cases of "status epilepticus," has been of much aid in my hands. The routine daily use of adrenalin in petit mal series and as a preventive measure for grand mal, however, has not proved of much benefit. I have had no experience with ephedrin.

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THE PROPHYLAXIS AND TREATMENT OF HAY FEVER AND
ASTHMA IN ROOMS MADE POLLEN AND DUST FREE
BY MEANS OF MECHANICAL FILTERS*

By MILTON B. CONYER, M.D., CLEVELAND, OHIO

IN JANUARY, 1924, one of my patients, sensitive to both grass and ragweed pollen, told me in detail the type of consulting engineering in which his company was engaged. He explained that they were keeping industrial plants dust free and were completely eliminating from the air fine dusts, such as lead or zinc oxide, the particles of which are less than 0.5 micron in size. I explained to him that pollen was dust and asked him if it would be feasible to eliminate it from the air of an ordinary room and to maintain the room pollen and dust free by means of some simple and comparatively inexpensive apparatus. He said he thought it could be done, and undertook to furnish me with an experimental apparatus. In March 1926¹ I published a preliminary report of the use of this machine at Mt. Sinai Hospital during August and September, 1925. In brief, it was determined that all pollen could be filtered out of the air of an incoming air stream, and that so much filtered air could be brought in each minute that a positive pressure of clean air was produced within the room, and no air could enter through the other cracks and crevices. Thus, it was not necessary to change the room in any way, other than to install the filter. Patients with simple hay fever cleared up within twelve to twenty-four hours, and those with asthma in two to five days, depending on the severity of the symptoms.

The first machine was very cumbersome, it measured 7' x 4' x 18" and was impractical for home use. Before the 1926 hay fever season it had been reduced in size so that it could be installed in any bedroom window, but it was still noisy. Six of them, however, were placed at my disposal for tests under home conditions. The results of these tests were published in *Clinical Medicine and Surgery*, April, 1927,² and substantiated in every way the observations of the preceding year. In fact, cases did so well that my patient decided to redesign the machine so as to make it commercially satisfactory. This has been done, and it is now available through all instrument supply dealers.

It consists of a fan, motor, and the filter material inclosed in a metal housing and mounted on a metal stand (Fig. 1). The intake is connected by means of adjustable metal pipe to an adjustable metal slide which rests in a window. The air is sucked in from the outside and forced through the filter material into the room at the rate of 200 cubic feet per minute. The filter material consists of several layers of a specially woven wool and cotton cloth, napped on both sides, backed by one layer of cotton cloth, napped on the in-

*Read before the American Association for the Study of Allergy, Washington, D. C.
May 17, 1927.

There is much clinical evidence to support the belief that there is a quantitative relationship between the amount of allergen necessary to produce hay fever and that which is necessary to produce asthma, also that patients vary in their sensitivity, and that amounts of pollen, for example, which will produce severe symptoms in one are innocuous in another. Pollen free rooms, together with pollen plate counts in the neighborhood, enable one to study these relationships accurately.

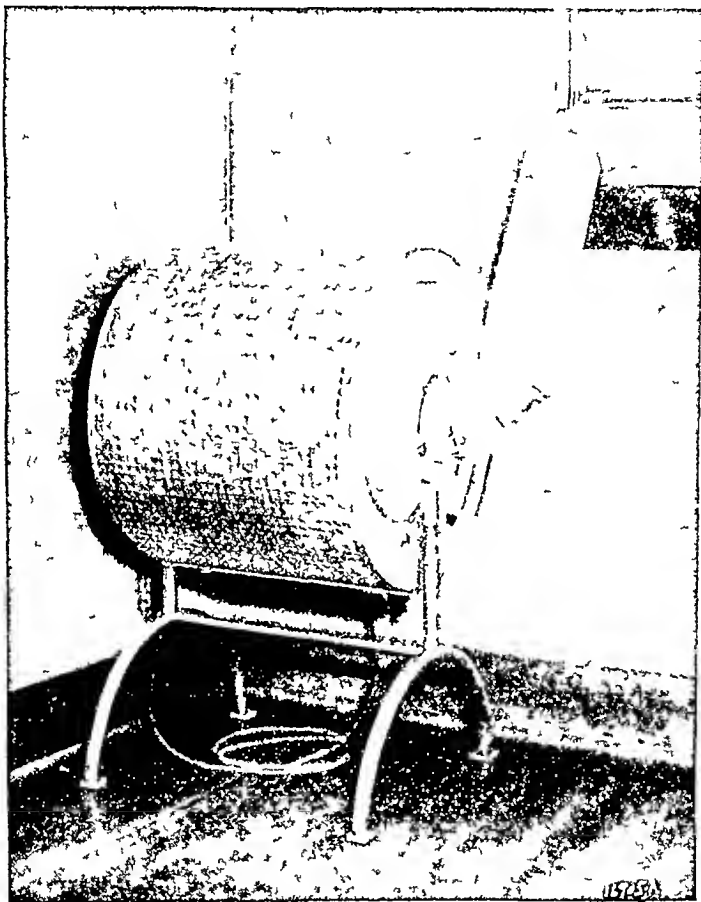


FIG. 1

In many sections of the country, particularly in the south and in the far west, there is a year-round pollen problem. Filtered air affords a method of obtaining relief for pollen cases in these localities while immunization is being carried out.

The following histories illustrate some of these points.

CASE 1—L. C., male, aged 34, has had perennial attacks of asthma since coming to this country from England 15 years ago. He had tried several climates without relief. When first seen by me he was completely debilitated. No definite sensitization could be demonstrated except, by hypodermic test, to the pollens of orchard grass, timothy and short ragweed. The chest showed a well marked chronic emphysema with marked bronchitis and mediastinitis. There was no demonstrable tuberculosis, either by roentgenologic or laboratory examinations.

Van Leeuwen⁴ thinks that all asthmatics are sensitive to air borne substances, many of them of unknown origin and composition. He has constructed so called miasm free rooms, ventilated by suction fans, which take the air from a height of 30 feet or more above the house top and filter it through cotton. His own home is completely ventilated in this way, the air being chilled before filtration to cause small particles to coalesce, so as to be more easily caught in the filters. He reports that asthmatics may be divided into two groups: one a group which remains asthma free when breathing miasm free air for ten to twelve hours daily and the other, a group which requires several days of continuous breathing of such air to become

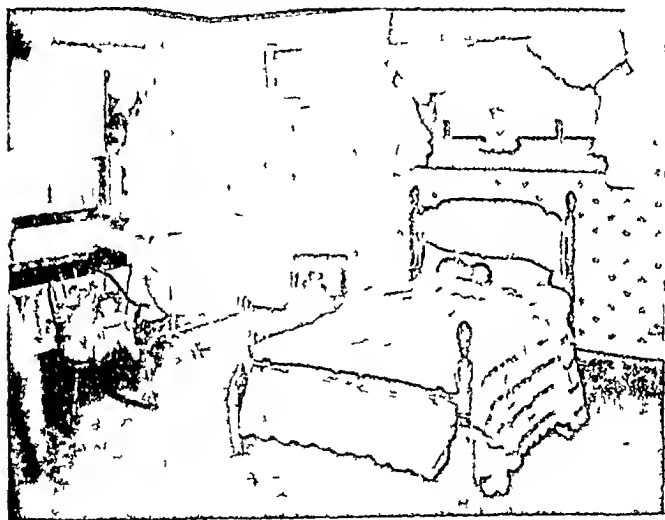


Fig. -

symptom free. Once free individuals in this group also may be outside for a variable number of hours daily without recurrence of symptoms.

These are the only references I have been able to find concerning air filtration for the prophylaxis and treatment of hay fever and asthma.

There are many problems presented by individual patients which the use of dust and pollen free rooms helps to solve. For example a patient reacts to several foods, an epidermal protein and to house dust. The foods are eliminated from the diet, the source of the epidermal protein is removed if possible, and the environment is rearranged so as to exclude as much house dust as possible. The asthma persists though in a mild form. A filter is placed in operation in the home, without further changes in the environment. If the asthma clears up, it has been due to some inhalant factor which has either been unrecognized or has not been eliminated from the environment.

There is much clinical evidence to support the belief that there is a quantitative relationship between the amount of allergen necessary to produce hay fever and that which is necessary to produce asthma, also that patients vary in their sensitivity, and that amounts of pollen, for example, which will produce severe symptoms in one are innocuous in another. Pollen free rooms, together with pollen plate counts in the neighborhood, enable one to study these relationships accurately.

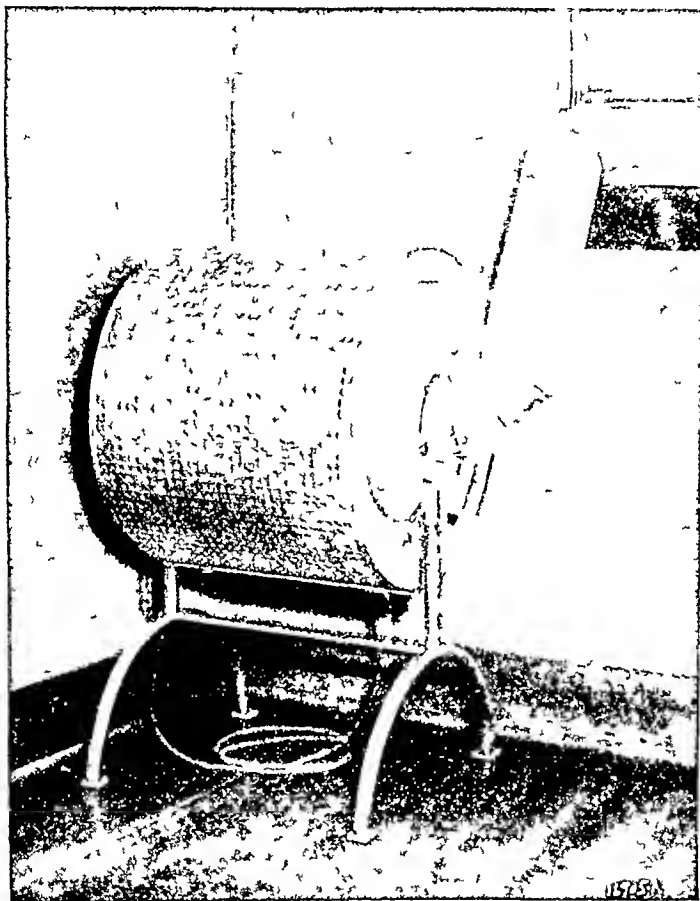


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In June 1925 there was an extremely severe exacerbation of asthma which lasted until about August 1. On August 18 following a ride in the country, very severe asthma again recurred and he was soon in an asthmatic state requiring eight to twelve injections of 5 minims of epinephrin each 24 hours to allow even difficult respiration. From August 18 until October 1 the attack never ceased. From October 1925 until May 15, 1926 there was gradual improvement though he was never able to return to work and required from 4 to 6 injections of epinephrin daily.

In June, 1926, an exacerbation due to grass pollen supervened. I saw him August 5, 1926 and on August 10 placed a pollen filter in his room and started it up. He has been in this room constantly except for the short time necessary daily to attend his natural functions. No pollen has appeared on plates exposed in this room. There have been no exacerbations of his symptoms and no epinephrin has been used.

CASE 2—N C woman aged 30 (the wife of patient I C mentioned above), has had fall hay fever for 10 years. On August 18 1926 symptoms began. She was advised to sleep in the pollen free room and to spend as much time there as possible during the day. During the first two days she averaged 18 hours daily in this room. Her symptoms disappeared entirely. She finds that she can be out from 4 to 8 hours daily without symptoms depending on the pollen concentration in the air on the particular day.

CASE 3—J C male aged 35 occupation physician has had hay fever without asthma every September for many years. He has usually planned a vacation to the Canadian fishing grounds during the hay fever season. Two years ago he had preseasonal immunization with ragweed pollen without relief.

Symptoms began about August 18 1926. On August 21 a filter was placed in his bedroom and one in his office so that he was able to be in pollen free air from 12 to 16 hours daily. He noted marked improvement when he remained 12 hours daily in filtered air and very marked improvement when remaining 16 hours. He has been able to remain at home with practically no discomfort for the first time in a number of years.

CASE 4—F L J male aged 50 occupation consulting engineer in dust collecting has had grass and ragweed hay fever and asthma every year for 18 years. The attacks totally incapacitated him so that he was usually unable to attend to his work until the middle of winter. Two years ago he spent the fall season in Europe and escaped his symptoms. Last year coseasonal immunization with grass pollen afforded relief from the early symptoms. Because of his business affairs only about half of the preseasonal ragweed immunization was completed.

He was perfectly well until August 21 1926 when a severe attack of asthma came on while he was in Detroit. The symptoms were very severe on August 30 despite hypodermics of epinephrin to the toxic limit every hour or two. On August 31 at noon a filter was placed in his bedroom window. Within 36 hours the acute attacks ceased there remained a hacking cough. On September 2 he went to his office for 3 hours but returned to the pollen free room, remaining over night and returning to his office the next day. At this time there were no symptoms.

For the next three days he remained at home in the pollen free room. Since September 6 he has been in the room from 14 to 16 hours daily and has been entirely free from symptoms except for one day when he experimented with himself and remained away from home for 16 hours. The hay fever which was severe that night had entirely disappeared by the next day.

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THE IMMUNIZATION OF HORSES TO ERYSIPELAS STREPTOCOCCUS TOXIN*

By JOHN F. ANDERSON, M.D., AND GEORGE F. LEONARD, M.D.
NEW BRUNSWICK, N. J.

ANTISTREPTOCOCCIC sera were used for the treatment of erysipelas as long ago as 1895, but until the work of Birkhaug in 1926, none of these sera had been standardized, and none had been concentrated. The results on the whole with these antistreptococcic sera were unsatisfactory, although some reported distinct therapeutic benefit following their administration.

In 1926, Birkhaug† announced the production of erysipelas streptococcus antitoxin prepared by the immunization of horses according to the method suggested by Dochez for the production of scarlatinal antistreptococcic serum. Birkhaug used the antitoxin so prepared, both concentrated and unconcentrated, for the treatment of cases of erysipelas. He stated that this antitoxin possessed very marked curative properties when administered early in the disease, and while the results were not so striking in late cases, nevertheless its use was of distinct benefit over other methods of treatment.

The results we wish to report embrace in part the immunization of 21 horses, the concentration of the plasma obtained from these horses, and the clinical use of the refined antitoxin in cases of erysipelas. We have used in connection with the immunization of our horses five strains of erysipelas streptococci furnished us by Dr. Konrad E. Birkhaug.

We have used various methods for the production of erysipelas toxin to be used for the immunization of horses, but have finally adopted almost as a routine procedure the use of horse meat broth containing 1 per cent peptone, 0.5 per cent sodium chloride and 1 per cent citrated horse blood. The meat broth with peptone and sodium chloride is made with a P_H value of 7.5 to 7.8. After being autoclaved, the citrated blood in the proportion of 1 per cent is added to the material before inoculation with the seed cultures. One thousand c.c. of the meat broth is put into 2000 c.c. Erlenmeyer flasks and the inoculated broth is incubated for 5 to 6 days, at the end of which time 0.5 per cent phenol is added. This is allowed to stand for 24 hours or longer, after which time it is either passed through a Berkefeld filter or through a Sharples centrifuge. After sterility tests have been made to insure the sterility of the toxin it is tested for the number of skin test doses in each cubic centimeter.

IMMUNIZATION OF HORSES

In our endeavor to determine the most satisfactory method for the immunization of horses, we have used various procedures. Some animals have been immunized by the use of toxin alone, others by the use of toxin and the

*Presented before the joint meeting of the Society of Immunologists and Association of Pathologists and Bacteriologists, Rochester, N. Y., April 15, 1927.

†Jour. Am. Med. Assn., May 8, 1926, p. 1411.

injection of whole broth cultures containing either dead or living streptococci, still others have been immunized by the use of the Zinsser Ginnel* blood clot method, or by a combination of the blood clot method and the injection of filtered or centrifuged toxin. We have found that from the beginning the horses stand the injection of comparatively large amounts of strong toxin and that the amount of toxin can be rapidly increased. Occasionally an animal is encountered which develops a marked edema following injection of even moderate amounts of toxin. We have tried the various methods mentioned above for the immunization of horses, and have adopted almost as a routine procedure the following method.

We usually begin with an initial dose of 20 c.c. of toxin and double this dose at each subsequent injection until the animal shows some reaction or until a volume of 300 or 400 c.c. is reached. In the beginning the horses are injected twice a week and this is continued for about three weeks, when the injections of toxin are reduced to once a week. Once a week the animals are also given treatment with the blood clot method and this combination is continued until the animals have been under treatment for approximately three months, when trial bleedings are made and tested for antitoxin content. The horses are usually bled 6 to 7 days after the last injection. The average time from the beginning of immunization to the first bleeding for production in our horses has been 100 days. The cultures used by us are highly virulent, and when treatment has been pushed too rapidly, particularly during the first six weeks of immunization, severe reactions have been encountered and even death has followed in a number of the horses. Reactions usually manifest themselves by extreme edema, high elevation of temperature, loss of appetite, and rapid emaciation. Autopsy of some of these animals has occasionally shown infective arthritis and in some instances endocarditis and the erysipelas streptococci have been isolated from such lesions. The plasma from the different horses has given an average test within three months after beginning of administration of 5000 or more neutralizing skin test doses per cubic centimeter. If the general condition of the horse remains good there is a gradual increase in the potency of the plasma under continued treatment although none of the animals seem to survive as long as animals that have been immunized with other toxins such as diphtheria or tetanus toxin.

PREPARATION OF THE ANTITOXIN

The horses are bled after trial bleedings have shown their plasma to be of a sufficiently high antitoxin content, not less than 6 days after the last injection, and the plasma after removal from the corpuscles is preserved with 0.5 per cent phenol. The plasma is concentrated by a modification of the Banzhaf method, using the methods we employ for the concentration of diphtheria, tetanus, and scarlet fever antitoxin. The average concentration of the plasma has given an antibody concentration of 4 to $5\frac{1}{2}$ times. The finished globulin has tested from 40,000 to 70,000 neutralizing skin test doses per cubic centimeter. The antitoxin after concentration and passage through a

*Proc. Soc. Exp. Biol. and Med. Dec. 1920, xxiii, p. 40.

Berkefeld filter is tested for the number of neutralizing skin test doses per cubic centimeter. It is distributed in doses containing 500,000 or more neutralizing skin test doses when tested according to the method used by Birkhaug.

We have received from the Dept of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y., standardized erysipelas toxin which we have used for the standardization of our erysipelas antitoxin. This toxin is diluted so that there is contained 1 skin test dose in each 0.1 c.c. injected. A dilution of the antitoxin is made, and the two are mixed and allowed to remain in the incubator at 37° C. for 1 to 2 hours, 0.2 c.c. of this mixture is injected intracutaneously into the upper third of the forearm of susceptible individuals. The tests are read after an interval of 24 and 48 hours and any reaction 1 cm. or more in diameter of the toxin-antitoxin mixture injection is considered a positive reaction and failure of complete neutralization of the toxin by the antitoxin.

CLINICAL USE

We have had reports on the use of this concentrated erysipelas antitoxin in widely separated sections of the United States, and it is clear that its use offers many advantages over the nonspecific treatment of the disease. All who have reported are impressed with the prompt improvement in the general condition of the patient, and confirm the statement of Birkhaug that the dominant clinical effect of the specific serum treatment in erysipelas is the prompt improvement in the toxic depression of the patient, followed by a rapid drop in temperature, pulse and respiration, sometimes observed 12 to 18 hours after injection of the antitoxin. When adequate doses of the antitoxin are given early in the disease, there is a rapid disappearance and fading of the erysipelas lesions and absorption of the blebs and pitting edema in previously affected parts. When given late in the disease the temperature and pulse usually decline by lysis, although amelioration of the toxic depression is just as distinct as when given early in the disease.

We have reports of 32 cases treated in one institution with concentrated erysipelas antitoxin. The average dose of antitoxin used in these cases was 20 c.c., each c.c. of which contained more than 50,000 neutralizing skin test doses. The minimum amount used was 10 c.c. and the maximum 36 c.c. Results of the treatment of the 32 cases with the antitoxin showed in the 24 hours an average drop in temperature of 3° F. and a reduction of the pulse rate by 30. The average time after the injection of the antitoxin until discharge from the hospital was 6 days, the minimum was 3 days and the maximum 12 days. Serum sickness developed in about 20 per cent of the cases.

ASTHMA IN CHILDREN VII COMPARATIVE METHODS OF SKIN TESTING WITH DIFFERENTLY PREPARED EXTRACTS OF HOUSE DUST*

By M. MUIR AND PESHKIN, M.D. New York, N. Y.

THE importance of house dust as an etiologic sensitization in asthma was stressed by Cooke in 1922. At that time I employed the scratch method only for determining skin sensitiveness to the various proteins in dry powdered form. A preparation of powdered dust extract was then made as follows: vacuumed house dust was extracted with a solution of 5 per cent sodium chloride for three days under toluol. The filtered extract was dialyzed against running tap water for from twenty-four to thirty-six hours, the dialysate concentrated ten times then filtered and the filtrate precipitated with acetone. The powder thus obtained was found to give positive scratch reac-

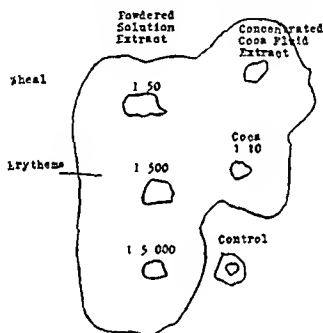


FIG. 1.—Comparative intradermal house dust reactions with the powdered solution extract and the concentrated Coca fluid extract (Case 4).

tions in 58 per cent of children with allergic asthma.¹ Sufficient powder was added to a solution of 0.8 per cent sodium chloride to make a 1:100 dilution which was rendered sterile after placing in a water bath for one hour at 58° C for two successive days. This solution was successfully used in the treatment of patients etiologically sensitive to house dust. Comparative scratch tests made with the dry powder and the 1:100 solution showed the former to give the larger number of positive reactions. Peshkin and Fineman,² in a study on 91 children with asthma, showed by comparative tests with the scratch method that the dry powdered house dust extract was positive in 42 per cent of the cases, the reactions ranging from plus minus to four plus, the concentrated fluid

From the Children's Asthma Clinic, Mt. Sinai Hospital.
Received for publication July 18, 1927.

extract (Coca) was positive in only 11 per cent of the cases, the reactions ranging from plus minus to one plus. The comparative scratch (dry powder) and the intradermal (Coca extract) tests, however, showed the intradermal method to be superior. Failure to obtain more and larger positive reactions with the concentrated fluid extract by the scratch method according to Coca,³ seems to indicate a low concentration of the house dust excitant in the fluid extract. Attempts to concentrate further these fluid extracts in some way in order to obtain a more potent preparation have thus far been unsuccessful.

With the foregoing thoughts in mind, the extracts of house dust used in this study were prepared from the same dust as follows:

1. Vacuumed dust was extracted with Coca's buffer solution for four days under toluol. The filtrate was concentrated at least ten times and dialyzed

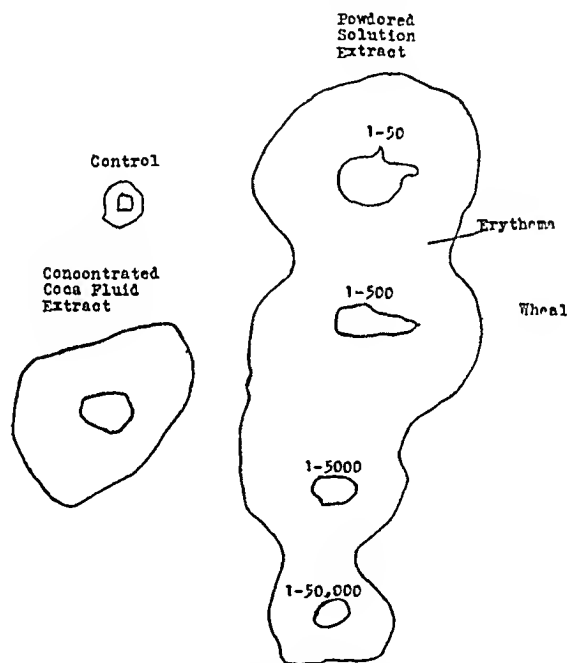


Fig 2—Comparative intradermal house dust reactions with the powdered solution and the concentrated Coca fluid extracts (Case 14)

against daily changes of buffer solution for seven days, toluol being employed as a preservative. The dialysate was filtered through paper. To one-quarter of the filtered dialysate, phenol was added up to a concentration of 0.4 per cent and the solution passed through a sterile Berkefeld filter. This solution was designated Coca extract.

2. To the remainder of the dialysate, three volumes of acetone were added and the precipitate, after being dried, was ground into a fine powder (Buffer solution is not precipitated by acetone).

3. Sufficient powdered extract was added to buffer solution to make a 1-50 dilution which was agitated daily for one week under toluol. More than one-half of the powder was not dissolved. This preparation was designated 1-50 powdered solution extract.

All three extracts were employed for making comparative scratch tests while the Coca and the 150 powdered solution extracts were used for the intradermal tests in patients known to be sensitive to house dust. The results of these tests are shown in Table I.

TABLE I

COMPARATIVE REACTIONS TO DIFFERENTLY PREPARED EXTRACTS OF HOUSE DUST BY THE SCRATCH AND INTRADERMAL METHODS OF SKIN TESTING

OASF NO	AGE	SEX	SCRATCH METHOD			INTRADERMAL METHOD†				
			150 CONCENTRATED			POWDERED SOLUTION				CONCENTRATED COCA FLUID
			POWDERED EXTRACT	POWDERED SOLUTION	COCA FLUID EXTRACT	150	1500	15000	150000	EXTRACT
1	1	M	+	±	±	15×17	14×16	10×12	9×7	12×14
2	5½	M	+	±	-	14×14	12×8.5	9×6	6×5	12×10
7	1½	M	+	-	-	12×14	14×10	10×10	6×6	10×10
4	8	M	+	-	-	15×7.5	10×7	8×6.5		8×6
5	8	M	±	±	-	15×11	12×6.5	9×8		9×10
6	8	M	-	-	-	12×15	11×8	6.5×7		10×7
7	9	M	+++	-	-	12×15	14×12	10×10	11×7	14×8.5
8	10	M	+	+	-	10×12	20×9	11×7	9×5	13×9
9	19	F	+	-	-	0×15	12×15	8×9		12×13
10	10½	M	-	-	-	10.5×9	11×8	7.5×6	5×4	10.5×8
11	11	F	+++	+	±	19×14	16×13	1×7	8×5	11×10
12	11	F	-	-	-	10×14	9×10	0×6	7×6	0×0
17	13	M	+	±	±	17.5×14	13×11	9×9		15×0
14	14	F	++	+	±	20×15	20×5	11×7	9×7	13×10

*The measurements for the scratch reactions (± to + + + +) are recorded in a recent publication¹

†The figures represent two diameters of the wheel in millimeters. The zones of erythema surrounding the wheals are not recorded. The controls ranged from 3 to 5 mm in diameter.

CONCLUSIONS

1 A solution of house dust more concentrated than the Coca fluid concentrated extract has been prepared from the dry powder and was successfully employed in the treatment of patients etiologically sensitive, in spite of the fact that more than one half of the powder does not go into solution.

2 A 150 solution of powdered house dust extract was found by intradermal test to be from ten to one hundred times more potent than the concentrated fluid extract of Coca.

3 Comparative reactions obtained by the scratch method with the dry powder, the 150 solution of powdered dust and the concentrated Coca fluid extract were positive in 78.50 and 36 per cent, respectively. The reactions with the powdered dust ranged from one to four plus, the 150 solution of powdered dust from plus minus to one plus and the Coca extract plus minus only.

The intradermal method for testing patients against house dust is superior to the scratch.

4 The foregoing suggests the concentration of other test substances which may prove to be of special value in those patients refractory by skin test to the present extracts.

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THE ACTION OF EPINEPHRIN UPON THE CARDIAC RHYTHMS*

By HAROLD L. OTTO, M.D., NEW YORK, N. Y.

ABNORMALITIES in rhythm are among the prominent objective indications of heart dysfunction or disease. The simplest classification of these abnormal rhythms is to divide them into two groups. The first, the homogenetic arrhythmias, comprises simple tachycardia and bradycardia and the various types of heart block, sinu-auricular and atrioventricular, from partial to complete, of the main stem of the bundle of His or one of its divisions, the second, the heterogenetic arrhythmias, contains the various types of premature contractions, the paroxysmal tachycardias and auricular and ventricular flutter and fibrillation.

The action of epinephrin may be epitomized as that of a sympathetico-mimetic, stimulating all sympathetic nerve endings. Its characteristic general circulatory reaction is an abrupt rise in the blood pressure consequent upon the vasomotor peripheral constriction†. This abrupt rise in the blood pressure produces reflexly, via the afferent depressor nerve, a sharp vagal stimulation. Its direct action upon the heart is complex—it produces increased rate and force of contraction, an irritable state of the myocardium which may induce a heterogenetic arrhythmia and an action upon the coronary vessels. The rate of rhythmic impulse production of the normal pace maker of the heart is greatly enhanced, the rate of the rhythmic impulse production of the atrioventricular node is likewise greatly increased if it is functioning as the pace maker for the heart, or if not, the potential rate of rhythmic impulse production of this tissue is increased to the proportionate degree, the tertiary centers for impulse production in the body of the ventricles are greatly heightened in tone, and this often appears in the production of premature contractions under comparatively normal conditions.

Under normal conditions of rate and rhythm, stimulation of the vagus nerves produces slowing of the rate or stoppage of the sinu-auricular node and the onset of nodal beats (escape) during the sinus inhibition. Types of heart block may appear particularly after stimulation of the left nerve, prolonged conduction time, dropped beats, or two to one heart block, effects of its inhibitory action at the atrioventricular node.

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†The vasodilator response that it also may evoke is usually an insignificant factor so far as its relation to cardiac phenomena is concerned. It appears most often late in the course of the action and is not great in degree.

With a powerful reflex vagal stimulation present under the conditions of rate and rhythm production as outlined for the intrinsic cardiac action of epinephrin, it is to be expected that many abnormalities in rhythm are capable of appearance. To point out that this is so and that epinephrin is capable of inducing practically every known type of arrhythmia is the purpose of this paper.

Since epinephrin is a sympathetomimetic, its cardiac action, apart from the vagal stimulation it induces which is a reflex conditioned by the abrupt rise in the blood pressure, must be a result of the alterations in rhythm inducible by the accelerator nerves to the heart. Dale¹ studying the action of an impure preparation of ergotoxin (chrysotoxin) which paralyzes the motor myoneural endings of the sympathetic nerves stated that the drug produced "a simple progressive abolition of the action of the sympathetic fibers and of adrenalin." On the heart of the frog ergotoxin abolished in equal measure the response to accelerator nerve stimulation and to epinephrin. Rothberger and Winterberg studying the action of the accelerator nerves to the heart found no distinction in the behavior of the heart response to epinephrin or direct stimulation of the nerve when the fact that epinephrin acts simultaneously on the endings of both nerves is considered.

Some minor differences in a strict comparison between the action of epinephrin and the accelerator nerves do however exist. Loewi² found in the heart of the frog that stimulation of the accelerator nerve is associated with the liberation of an augmentor substance which resembles epinephrin in its action, and Witanowski³ that abolition of the response of the heart of the frog to accelerator stimulation precedes the abolition of the response to epinephrin. I have observed the same phenomenon in the mammalian heart.* Lundberg⁵ concluded from the results of his study with hydrastinin that epinephrin acts not only on the end organs of the accelerator nerves but also on the heart muscle directly.

THE HOMOEOMETIC RHYTHMS

Epinephrin injection or accelerator nerve stimulation can raise the sinus rhythm in animals to rates as high as 330 per minute when the vagi are cut or paralyzed. I have constantly seen rate increases from 150 or thereabouts to 270 in cats and dogs by either epinephrin administration or accelerator nerve stimulation (usually the right nerve) in the same animal. It is not rare to observe a greater acceleration following stimulation of the nerve than after epinephrin. The majority of animals give equal responses to both when maximal responses are studied. This is also true for impulse production arising at the atrioventricular node excepting that under the same conditions the rate responses are lower. With intact vagi, nodal rhythm is almost invariably present during the first period of response to epinephrin.

The intravenous injection of small doses of epinephrin in man gives similar results. With previous atropinization the heart responds to small intravenous doses of epinephrin with very high rates and in young healthy subjects small doses of epinephrin intravenously very commonly result in a short period of nodal rhythm.

When epinephrin injection produces heart block it is most usually due to the concomitant vagal stimulation and since this is an indirect effect of the epinephrin action it is not to our purpose to enter into detailed discussion. Kahn⁶ has reported heart block action in dogs after injection of epinephrin, and Clough⁷ has reported the temporary appearance of delayed conduction and partial heart block following the intramuscular exhibition of 0.5 cc of epinephrin in cases of so called irritable heart.

THE HETEROGENETIC RHYTHMS

There is abundant evidence in the literature that epinephrin will induce premature contraction. Kahn⁶ reported premature contractions in dogs after injection of epinephrin, Danicopolu and Danulescu⁸ reported the appearance of premature contractions after injection of epinephrin in man. Smith and Moody⁹ reported the appearance of ventricular premature contractions following the subcutaneous injection of 0.6 cc of epinephrin in an individual predisposed to them. DeGhaff and Weiss¹⁰ observed the spontaneous appearance of ventricular premature contractions and the increase in their number when they were already present after the subcutaneous injection of 1 cc of epinephrin in complete heart block in man. Clough⁷ reported the temporary appearance of ventricular premature contractions in cases of so-called irritable heart following the intramuscular injection of 0.5 cc of epinephrin. Otto and Gold¹¹ in a clinical study of the persistent premature contraction pointed out that 1 cc of epinephrin in subcutaneous injection never failed to cause a considerable increase in the number of premature contractions prevailing at the time, were they auricular or ventricular in type, and patients with auricular premature contractions frequently developed ventricular premature contractions and vice versa, the same being true of right or left premature contractions. It was not uncommon to observe short runs of serial premature contractions in very fast rhythm, which is the transition stage to a heterotopic tachycardia.

The rôle played by the vagus under these conditions is a passive one, in that the reflex vagal stimulation following the rise in the blood pressure depresses the rate of the sino-auricular node and thereby permits the excitation of the lower centers to evince itself. This was pointed out by Nobel and Rothberger¹², when the high rate of the upper centers is allowed full sway the premature contractions occur in greatly diminished number if at all. In 5 dogs of a series of 20, in which no other procedure than the intravenous injection of epinephrin induced many ventricular premature contractions, section of the vagus or atropinization caused a great reduction or abolition of the premature contractions excited by the epinephrin*. The appearance of the premature contractions is the effect of accelerator nerve stimulation. Rothberger and Winterberg² found it to be a practically constant phenomenon in their experimental series. That removal of the influence of the vagus has no effect upon the premature contraction unless it causes a considerable increase in the rate, has been pointed out by Otto and Gold¹¹. Of eight patients with persistent premature contractions atropine had no effect upon them ex-

*Unpublished experiments

cept in one patient in which there was a great increase in the rate, and this effect endured only as long as the high rate continued. Gold and Otto¹¹ studying bigeminy found that atropine abolished the premature contractions only while the rate was high.

PAROXYSMAL TACHYCARDIA

Kahn was able to induce paroxysms of tachycardia in dogs with the aid of epinephrin. Levy¹² reported multiple tachycardia as a constant effect of epinephrin in cats under light chloroform anesthesia. That the reflex vagal stimulation induced by the epinephrin was an important factor, favoring the appearance of the arrhythmia, was pointed out by Nobel and Rothberger.¹³ They further demonstrated that this action of the vagus was the depression of the activity of the upper centers for impulse production, which thereby gives the already excited lower centers fuller expression. Danileopolu and Danulescu⁴ reported an instance of epinephrin inducing short paroxysms of auricular tachycardia in man.

Otto and Gold¹¹ reported a study of paroxysmal tachycardia in a cardiac patient who had never failed to get a paroxysm of auricular tachycardia after the subcutaneous injection of epinephrin when not previously subjected to the influence of other drugs. These paroxysms were indistinguishable from the spontaneous paroxysms to which the individual was subject in symptoms, rate, duration and electrocardiography. This was an effect of accelerator nerve stimulation in which the vagus nerve played an etiologic role since the preliminary exhibition of atropine prevented the onset of tachycardia. The exact nature of the reflex vagal action in this auricular tachycardia was not determinable.

Stimulation of the accelerator nerves or epinephrin injection never fails to induce a ventricular extrasystole tachycardia in dogs in which the tertiary centers for impulse production are sensitized by very small doses of barium, as Rothberger and Winterberg have pointed out and the stimulation of the vagus nerve favors this only by depressing the upper centers and thereby facilitating the expression of the autonomy of the lower. Similarly the barium has apparently no other role than that of raising the excitability of the tertiary centers.

Hence there is good and sufficient evidence that ectopic tachycardias are producible by epinephrin administration or accelerator nerve stimulation.

FLUTTER AND FIBRILLATION

In the laboratory it is commonly observed that auricular or ventricular fibrillation follows an injection of epinephrin. Smith and Moody⁹ reported two cases in which the subcutaneous injection of epinephrin 0.6 cc induced auricular fibrillation approximately 15 minutes after the injection. In one of these individuals the auricular fibrillation so induced endured for 2 hours before the normal sinus rhythm supervened.

We have in our records⁶ the clinical history of a male patient with arteriosclerotic heart disease subject to spontaneous attacks of paroxysmal auric

¹ summary of this case has been reported by me in the Proceedings of Society Experimental Biology and Medicine. A detailed report is to be published.

ular fibrillation, who twice during periods of normal sinus rhythm developed auricular fibrillation after subcutaneous injection of epinephrin which endured until the intervention of other drugs re-established the normal sinus rhythm. That the vagus played an etiologic rôle in this response was indicated by the fact that the previous administration of atropine prevented the onset of the auricular fibrillation after epinephrin injection.

The intravenous injection of very small doses of epinephrin very frequently induces auricular fibrillation in elderly normal individuals, and the previous administration of atropine will prevent its onset.

This is analogous to the induction of auricular fibrillation in dogs by the simultaneous stimulation of vagus and accelerator nerves (Rothberger and Winterberg²).

Levy¹³ has reported the constant appearance of ventricular fibrillation in cats under light chloroform anesthesia after small doses of epinephrin. The same author has also reported a similar effect following stimulation of the accelerator nerves, particularly the right nerve.

Rothberger and Winterberg² had a not inconsiderable number of their dogs develop ventricular fibrillation after stimulation of the left accelerator nerve.

I have had the unfortunate experience of observing, during the administration of an epinephrin series to a patient with acute pulmonary edema and a regular pulse of a rate of 140, sudden death shortly after the third injection of the series. The pulse slowly decreased in rate, to 90 per minute, five minutes after the third injection, becoming fuller and stronger as it decreased in rate with a corresponding improvement of the dyspnea. At this time premature contractions appeared which increased in number and occurred in groups. Upon this there occurred a long series of rapid and regular beats which ended by sudden and complete disappearance of the pulse and heart sounds. This was probably ventricular premature contractions, tachycardia, and fibrillation.

COMMENT

Since epinephrin by virtue of the powerful accelerator stimulation it induces can cause the appearance of cardiac arrhythmia, in many instances of a dangerous or unpleasant nature, it is distinctly advisable to administer it with caution particularly to cardiac patients or those individuals presenting a history of having had one of the cardiac arrhythmias.

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PATHOGENIC GIARDIASIS IN CHILDREN*

By M G PITTMAN M D MILWAUKEE WIS

THE pathogenicity of *Lambia intestinalis* or *duodenalis* (*Giardia*, *Cercaria* *monas intestinalis megastoma entericum*) is still doubted by many physicians. Many recent pediatric textbooks fail to mention at all the possibility of *Giardia* as a cause of enteritis¹ or mention the parasite as probably non pathogenic. The literature has been well reviewed by Lyon and Swalm² and quite recently by Zahorsky and McLoon.³

During 1926, 112 complete stool examinations were made in the Milwaukee Children's Hospital. In seven children intestinal parasites or their ova were found. *Lambia intestinalis* were found in four children. *Taenia solium* in one, *Entameba coli* in one and *Trichomonas intestinalis* in one. Of the four children infected with *Giardia* two presented intestinal symptoms.

C K, aged four years was brought to the hospital for treatment of multiple burns of the body. The family and past history were unimportant. There was no previous history of intestinal trouble. The child was in the hospital 32 days making an excellent recovery when he became restless irritable and lost his appetite. The stools which had been normal and passed one or two times daily became soft green and very offensive in odor. Examination of the stools showed numerous cysts of *Lambia intestinalis*, pus, mucus, and undigested food. The blood count showed erythrocytes 4 160 000 hemoglobin 70 per cent (Dare), leucocytes 21 050 of which there were 55 per cent polymorphonuclear neutrophils, 44 per cent lymphocytes 1 per cent large mononuclears and no eosinophiles. The stools increased to three to six daily they were soft sometimes liquid and yellow or green in color. After a few days the cysts became less numerous and large numbers of the flagellate forms of *Lambia* were found. These parasites were actively motile, pear shaped, with the sucking disc and dumb bell nucleus as described. Stovarsol (acetarson) was administered in one grain doses by mouth daily before breakfast. Four doses were given. On the second day after the drug was begun there was one small yellow stool containing few cysts and no flagellate forms. On the third day there was one soft, formed, brown stool. There were five formed brown stools on the fourth day. These contained few cysts no pus no mucus and no undigested foods. On the second day the child's appetite and disposition improved markedly. There were one to three normal stools daily with a few *Lambia* cysts to the time of discharge from the hospital. There has been no recurrence of enteritis.

R G, aged 13 years was brought to the hospital because of abdominal pain. The family and past history were unimportant. There was no previous history of intestinal trouble. Examination revealed a moderate tenderness over the epigastrium but no rigidity. The temperature was normal. The leucocyte count was 12,800 with 63 per cent polymorphonuclear neutrophils, 37 per cent lymphocytes, and no eosinophiles. The stools were

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soft, and sometimes watery, brown, yellow, or green and contained numerous cysts of *Lamblia*, pus cells, and undigested food. No flagellate forms were observed. The patient left the hospital before treatment was instituted and returned two days later with epidemic parotitis. During the second hospital stay the stools remained as above and the patient complained of abdominal discomfort, fullness, anorexia. No further cause for his symptoms could be demonstrated. He was again taken home before treatment.

The incidence of intestinal parasites is not high among our hospital and dispensary population. While the stool examinations are not numerous, they include all patients with any symptoms possibly caused by intestinal parasites. In 112 cases parasites were found in 63 per cent. *Lamblia intestinalis* were found in 36 per cent. In two children there was no evidence of intestinal disease. In one case *Lamblia* could be considered the direct cause of enteritis and in another a most likely cause of indefinite abdominal distress.

The habitat of the *Lamblia* parasite is considered to be in the duodenum and jejunum. The active flagellate organism is found only in acute cases in which the stool has been rapidly passed through the intestines. *Lamblia intestinalis* or *Giardia* must be considered at times pathogenic in children. The diagnosis is easily made by stool examinations.

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LABORATORY METHODS

A PRACTICAL METHOD OF MAINTAINING INDUCED POLLEN IMMUNITY *

By I S KAHN M.D., SAN ANTONIO TEXAS

IT HAS been my experience that in most instances, with but a small percentage of exceptions induced pollen tolerance is of only brief duration, commencing to diminish as early as the fifth or sixth day following its production, the diminution within a few weeks reaching the zero point as far as protection of clinical value is concerned. In communities where such protection has to cover a pollination season of only a few weeks' duration the attainment of the maximum dose at the onset of such pollination, with no further treatment may be clinically sufficient to hold symptoms in abeyance. In communities such as San Antonio with at least three pollens in the air for from four to ten months recurrence of symptoms is the rule within a few weeks after the cessation of pollen extract injections, and protection must be maintained.

The following method which is routine in my office has proved invariably successful in maintaining this protection and is recommended for use in cases where the annual preseasonal treatment is not practical or where protection has been technically difficult to secure. By this method treatment of any pollen can be started at any time of year. Also the uncertainty of reaching a sufficiently high protective preseasonal treatment dosage in succeeding years can be obviated. It is assumed that the maximum protective dose has been reached, and all local reaction at the site of treatment injections to such dose has been obliterated. It has always been my belief that as long as such local reactions persist, desensitization, while often clinically satisfactory, is not complete for such dosage. If this maximum dose be repeated every six or seven days, protection remains and local reactions do not reappear. If this interval be lengthened, local reactions reappear indicating a loss of tolerance. The return of reactions under such circumstances calls for shortening the interval and temporarily reducing this dose. In only a few instances have I been able to increase this interval beyond a week though I have one case clear for three years whose injections are given only every three weeks. Patients can be easily taught and persuaded to give themselves such treatment once a week.

I have seen no harm from this constant pollen treatment, though I have no patient carried thus far over three years. I have seen no indications that continuance of such injections for years longer will do any harm.

This method can be modified to the advantage of reducing bulky dosages and saving material following the close of the pollination season by holding the protection by a weekly dose of one-tenth to one-fourth of the maximum protective dose, running up the treatment a few weeks before the onset of the pollen season

THE USE OF A RESISTANCE THERMOMETER FOR RECORDING THE BODY TEMPERATURE

BY BURGESS GORDON, M D, AND E VON STANLEY, B S, PHILADELPHIA, PA

MEASUREMENT of the body temperature at intervals of less than one hour is often desirable but rarely attempted because it is time consuming for the nurse and disturbing to the patient. The resistance thermometer, which has been used satisfactorily at the Russel Sage Institute¹ and by Varrier-Jones in England,² for measuring the deep body temperature by rectum, affords a means for obtaining a record of the temperature without these objections.

The essential parts of the thermometer are

- (1) A coil of wire with a high temperature—coefficient of resistance
- (2) A Wheatstone bridge to measure the resistance of the coil
- (3) A millivoltmeter to indicate changes in potential across the bridge

The potential across the bridge obviously depends on the resistance of the coil, which in turn is a function of the temperature. By calibrating the millivoltmeter directly in degrees Fahrenheit this apparatus becomes a precision instrument for measurement of temperature.

A commercial type, recording, resistance thermometer (Fig 1) was modified for clinical use. A bulb (the heat sensitive portion) was designed for obtaining the rectal temperature. This bulb (Fig 2) is a silver tube, 6 mm in diameter and 72 mm long, containing a coil of nickel wire in the closed end. The other end of the bulb is attached to a soft rubber tube, 5 mm in diameter and 87.5 cm long, which carries three stranded copper leads imbedded in rubber. These terminate in a connector block.

The temperatures indicated on the millivoltmeter (temperature range extends from 85° up to 110° Fahrenheit) are recorded every 30 seconds on a special chart (Fig 1 C). The lag in recording does not exceed two seconds. The variations in the temperature may be read within one-twentieth of a degree.

The leads from the recording part of the instrument which connect with the bulb are carried in a secure flexible cable. This is made in sections of convenient length for reaching the beds. A simple adjustment balances out all lead resistance so that the accuracy and sensitivity of the instrument is

*From the Medical Service of Dr. Thomas McCrae and the Department for Diseases of the Chest, Jefferson Hospital, Philadelphia, Pa.
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unaffected by length of leads or by local temperature conditions at any point other than at the bulb. Portability has been secured by mounting the machine on a board equipped with lugs, so that it may be set up readily on any wall which has been prepared to receive the lugs.

After adjustment of the instrument, which requires no special training, the recorder is put in motion. A suppository, containing ethyl aminobenzoate (USPX) is inserted in the rectum and in about one half hour the bulb is

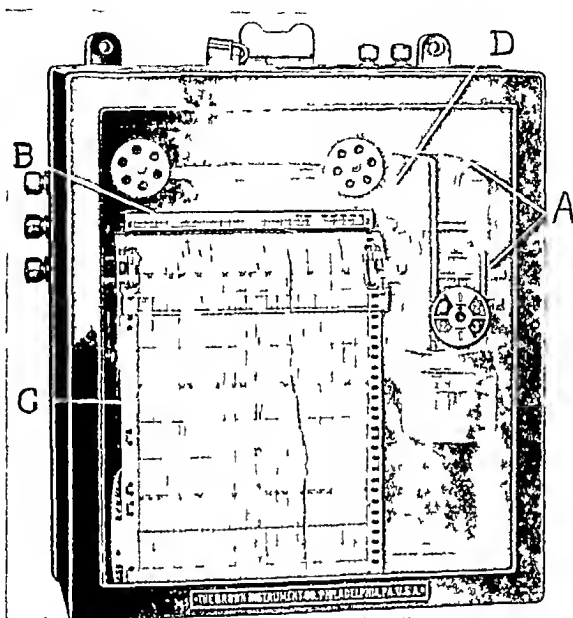


Fig 1—Recording resistance thermometer showing clock work. A indicator dial B chart C and printing apparatus D. The temperature scale runs horizontally and is read from left to right. The time scale runs vertically and is read upward. The temperature line is a series of small dots.

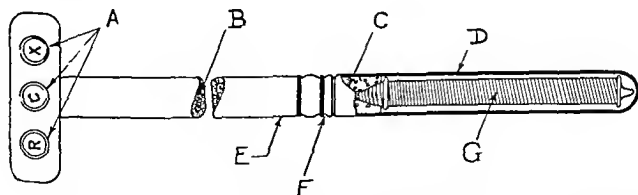


Fig 2—Section of bulb and cable assembly. Blinding posts A. 3 conductor cables B. cement C. silver protecting tube D. flexible rubber tubing E. connection ferrule F. sensitive resistance coil G.

passed beyond the anal sphincter. The bulb may be retained in the normal rectum for periods up to twelve hours, usually without discomfort, for one or more days. There is no danger of electrical shock since the thermometer operates on six volts 32 milliamperes.

The instrument has been found to be of value in accurately determining temperature variations in critically ill patients and in those who have sudden fluctuations of temperature.

The Apparatus was manufactured by the Brown Instrument Company, Philadelphia through the cooperation of Mr. W. H. Lukens.

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A SIMPLE METHOD OF KEEPING STERILE SERA AND FILTRATES DURING THE TEST PERIOD AND OF BOTTLING BY GRAVITY*

BY JOSEPH P. SCOTT, D. V. M., MANHATTAN, KANSAS

THIS method of holding and bottling sterile filtrates after passage through Mandler filter candles was developed at the Vaccine Laboratories of the Kansas Agricultural Experiment Station in order to provide a cheap, efficient, and simple means of testing large amounts of such filtrates and of bottling them from the original container after passing various tests.

The complete apparatus is shown in Fig. 2. It consists of a one gallon or two gallon E. Z. seal jar, a bottling top, two funnels, a filling tube, and an air filter.

In Fig. 1, the apparatus is seen in part, all the parts here shown are attached together and placed on the gallon jar which is not shown. The metal bottling top *T* is a casting from a mold of the ordinary glass lid that comes with these jars. Four tubes of various sizes are inserted in holes bored through this top. The air tube *a* is a small bore tube which extends to within an inch of the bottom of the jar when the top is in place and carries the air above the surface of the liquid being bottled. The filling tube *B* is a short large bore tube which is connected with the filter candle by means of a large bore rubber tube 2. Tube *C*, the bottling tube, is of the same size as *B* and is connected with filling funnel 3 by means of a rubber tube. The testing tube *D* is a small tube connected with test funnel 4 by means of two rubber tubes and a glass connecting tube 5.

The air filter 1 is a small glass tube filled with absorbent cotton. It filters the air that is carried by tube *A*. Rubber tube 2 is a short piece of heavy

*Contribution No. 37 from the Veterinary Department, Kansas State Agricultural Experiment Station.

This method was developed under the direction of Dr. L. W. Goss, Professor of Pathology, Ohio State University, while he had charge of blackleg research at the Kansas Agricultural Experiment Station.

large bore rubber tubing that slips easily and firmly over the filling end of the filter candle. Funnels 1 and 4 are aluminum and a glass tube passes through the neck of these funnels. This glass tube extends half way down the body of the funnel, and a swelling on the tube prevents it from slipping through

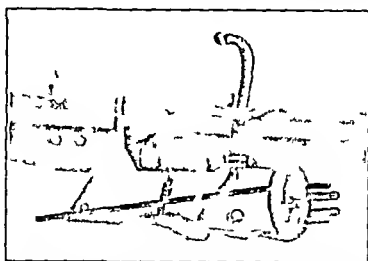


Fig 1—T bottling top 1 air tube 2 filling tube 3 bottling tube 4 test tube 5 air filter 6 filling tube 7 bottling funnel 8 test funnel

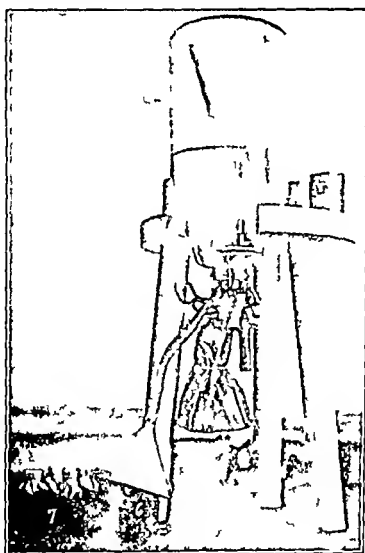


Fig 2—T bottling top A air tube B filling tube C bottling tube D test tube 1 air filter clamped and wrapped 2 filling tube clamped 3 bottling funnel wrapped 4 test funnel 5 glass tube joint 6 test bottle 7 test bottles from previous jars 8 metal weights 9 wooden tripod 10 gallon jar

the neck of the funnel. These funnels are attached to the tubes C and D by means of rubber tubing passing over the glass tube and the neck of the funnels. The filling funnel 3 is connected directly, the test funnel indirectly

by means of glass tube 5 which enables the easy removal of this funnel after the test samples have been taken

Before filtration of the material, the jar, bottling top, and other parts are assembled—all free ends are wrapped in separate pieces of paper—and the whole bottling jar is sterilized under at least fifteen pounds pressure for one hour. Following this sterilization the bottles are cooled, and filling tube 2 is slipped over the filling end of the filter candle. The material is then forced through the candle by pressure. As soon as the container is filled,

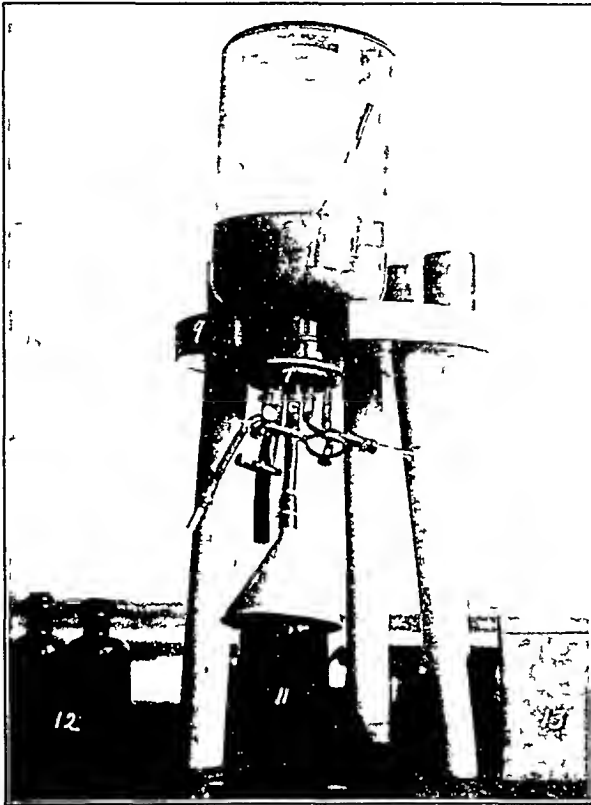


Fig 3 —T, bottling top. A air tube B filling tube C bottling tube D test tube 1 air filter 2 filling tube clamped 3 bottling funnel with spring clamp, glass tube joint clamped 4, mental weights 5 wooden tripod 6, gallon jar 7 500 ml serum bottle 8, filter bottles 9 bottle box

tube 2 is clamped off and slipped from the filter candle. All tubes are clamped with spring or screw clamps, and the jar is inverted on stand 9, Fig 2

Fig 2 shows the inverted jar 10 and the test being run from test funnel 4 into sample bottle 6. The stand 9 is a wooden tripod in which a circular notch has been cut, this notch serves to catch the neck of the jar. Weights 8 are placed on the back of the stand to balance the weight of the filling jar. In this figure the paper has been removed from test funnel 4 and a sample of filtrate is being taken off into test bottle 6. Two test bottles are taken from each jar 7, one culture flask test is also inoculated from each jar. After

taking all necessary samples test funnel 4 is disconnected at the glass tube 5, and the filtrate is then held in the ice box until the sterility tests have been made. The sterility tests are made on glucose broth, two fermentation tubes being inoculated from each test bottle. These tubes and the culture flask are incubated for seven days and if they prove sterile, the filtrate is then bottled into appropriate serum bottles through bottling funnel 3.

Before bottling, the bottles are placed in a metal box having a tight lid, the bottles are placed mouth down on several layers of cheese cloth or muslin which covers the bottom of the box. The boxes are then sterilized for at least one hour at fifteen pounds steam pressure.

Fig 3 shows the process of bottling. Air filter 1 is shown after the removal of its paper cover and clamp. This allows the free passage of the filtrate through filling funnel 3. The filtrate is seen passing into a 500 ml serum bottle 11. After being filled these bottles are corked 12 and later the sealing wax is added and the labels are placed on the bottles.

This method has been used to put up all kinds of liquid vaccines and gives a very convenient way of removing samples and holding the bulk of the vaccine until the appropriate tests have been made. It could also be used to retain and distribute special culture media which cannot be subjected to sterilization by heat.

The gravity method is simpler and more economical than the old syphon method which requires from 5 to 10 times the amount of rubber tubing.

A SIMPLE HOOD FOR USE WITH BINOCULAR MICROSCOPES*

By H. W. WADE, M.D., CULLION LEPER COLONY, P. I.

OF RECENT years attention has been paid the fatigue and consequent reduction of efficiency involved in prolonged use of the microscope. It can hardly be gainsaid that microscope fatigue is important, varying in degree with individual susceptibility but probably affecting most workers to some extent and some workers very markedly. Therefore any practicable measure for its reduction is of value.

The binocular microscope is decidedly superior to the monocular instrument in important features for the eyes are employed equally, the light reaching each eye is of reduced intensity, and the effort involved in inhibiting impressions other than those from the microscopic field is greatly reduced. This last factor is, however, by no means eliminated for a considerable amount of extraneous light still reaches the eyes from beyond the field of the ocular rim. To the writer at least this light seems conspicuous, and in bright weather it is decidedly annoying.

The binocular microscope certainly aggravates one fatigue factor, that

*From the Pathological Section, Cullion Leper Colony, Philippine Health Service. Published with the permission of the Director of Health.

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of posture, as is pointed out by Exton¹ in describing an apparatus in which the microscopic field is examined indirectly by means of a projected image. This aggravation is due to the necessity of holding the head continually in a more precisely orientated position than is required in monocular work.

Primarily to eliminate the incidental extraneous light, I applied a simple hood to a binocular eyepiece used in this laboratory. This hood has been used for several months with marked satisfaction, for besides practically eliminating this annoyance it has other advantages not anticipated, the most valuable of which concerns posture fatigue.

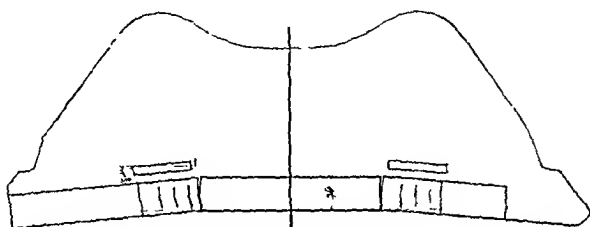


Fig 1—Diagram of hood as adapted to the binocular eyepiece shown in Fig 2 (Cross sections inch scale)



Fig 2—Hood in position for use

The hood, which is inexpensive and simple to construct, is adaptable to various makes of the more usual type of binocular apparatus used with single objectives, except, perhaps, when these are fitted with shutters under the oculars. Though the pattern here shown (Fig 1) is for a binocular eyepiece attachment, only minor changes are necessary to adapt it to the usual binocular tube.

CONSTRUCTION

Materials—1 A strip of thick cardboard (book-binders' board, or ordinary card built up as required), $\frac{3}{4}$ by 11 to 12 inches and as thick as the top of the prism case. 2 A piece of hard, stiff fiberboard is being used that from a "Fiberstok" filing envelope, but a slightly thicker jar 7, one would perhaps be preferable. 3 Black texiderm or other

similar water proofed book binders' material, somewhat more than one square foot 4 Cross section paper (a convenience)

PROCEDURE

1 Fit the strip of binders board as a collar neatly about the prism case, flush up against the projecting top. Cut to permit bending at corners and elsewhere as needed for the curved ends. Turn down at the ends if the projection is less there than elsewhere, as in the apparatus at hand. Fix temporarily in place with staples.

2 Cut from heavy paper a pattern of the general form shown in the diagram (Fig 1). If for the regular binocular microscope tube it will probably need be of greater height. Heavy cross section paper of inch scale is convenient for this. Modify to fit the microscope and the head and face. The angle at α is determined by the amount of flare desired. The slots for the sliding plates that carry the oculars are best cut after this is done. They should be large enough to allow for turning in the covering material.

3 Cut the fiber board to the corrected pattern and with a strong adhesive affix to the collar still on the microscope. When firmly set, remove and lay flat, cutting the collar where necessary for this.

4 Cut two pieces of the covering material. To allow for turning in, one should be about $\frac{1}{2}$ inch larger than the pattern except at the bottom edge where the excess should be nearly an inch to cover the inner surface of the collar. The other is cut to the pattern, or a trifle scant except at the bottom where it is merely to abut on the collar.

5 Assemble, using liberally a good library paste or other adhesive that does not dry rapidly. Apply the outer (larger) covering piece to the fiber board base, rubbing thoroughly into contact. Turn in the margin, clipping and crimping or goring where required. Cut the material through the slots as indicated by dotted lines in Fig 1 and carefully turn in the narrow margin then available. Apply the lining without cutting the slots.

6 After pressing under a weight until good adhesion is obtained but while the whole is still damp and flexible fit to the microscope. First, however, slit the lining longitudinally over the slots and after trial trim out in such a way that light is excluded. Mould the sides to fit the face. Allow to dry in place, to insure that the farther (forehead) margin of the top be flat, this may be clamped between two ordinary glass slides held by clips.

7 The hood shown in Fig 2 is held in place by an encircling tape (as a shoestring, here shown white for demonstration), which insures firm contact. Other methods are possible. A slight modification of arrangement is required for the regular binocular tube.

Use of the hood described is advantageous in several respects. If properly fitted to the user it eliminates incidental light almost completely. This not only minimizes fatigue of the retina but permits restful relaxation of the muscles of the eyelids, which automatically contract in consequence of such light. There being no conflicting light, the microscopic image stands out with greater crispness and apparent definition. Concentration on the work

in hand is facilitated by elimination of visual perception of movements in the room about one. An important feature is that the shield automatically maintains proper orientation of the head, this and the support obtained by even light contact of the forehead with it markedly relieve fatigue of the muscles of the neck. Adjustment of interpupillary distance is interfered with scarcely at all.

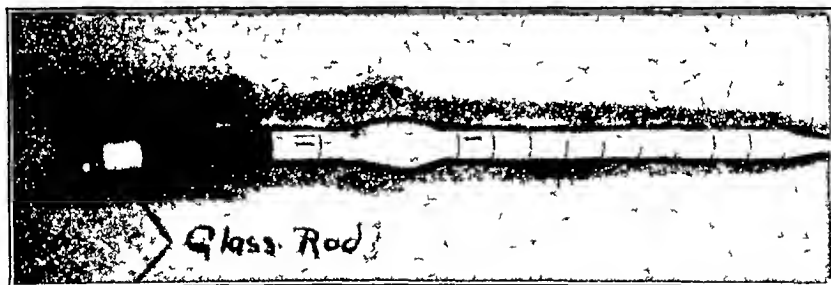
REFERENCE

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SIMPLE SEAL FOR BLOOD COUNTING PIPETTES*

BY ROBERT C. SCHLEUSSNER, M.D., NEW YORK, N. Y.

IF A SHORT length of the rubber tubing used with blood counting pipettes is tied at one end and the open end is slipped over the broad extremity of a filled pipette, it will be found that the air in the improvised cap expels the liquid as the cap is pushed into place. For the cap to function correctly it is necessary that there be some way of allowing the air in the cap to escape as the cap is pushed down over the end of the pipette. This is easily provided by



adapting the dropping device of the old style Mohr burette to our present needs.

The accompanying diagram shows the device. A short length of glass rod is slipped into a bit of rubber tubing about one and a half inches long. The rubber tubing should be of a diameter sufficient to fit very snugly over the end of the pipette, while the glass rod should be of a diameter sufficient to slightly distend the rubber tubing.

The device is used in the following fashion. After the blood has been sucked into the pipette in the usual fashion and the usual diluent added, the rubber tubing used for suction is removed while keeping one finger over the tip of the pipette to prevent leakage. With the pipette in the one hand, finger over the tip, the other hand slips the sealing device over the broad end of the pipette. As the rubber tubing is forced over the end, pressure is made over

*From the Lenox Hill Hospital Medical Service of Dr. O. Schwerdtfeger.
Received for publication March 7, 1927.

the glass rod in the tubing. This produces a minute air channel between rubber and glass and allows air to escape as the tubing is pushed securely into place. Now that the tubing is in place the pressure about the glass rod is released and the rubber tubing clamps down around the glass making an air tight joint. No matter which way the pipette is tilted no fluid will run out. Where the filled pipette is to be carried about in a bag, it is advisable to fix a seal over the pointed end as well as over the blunt end.

The sealing device may also be incorporated in the tube used for suction, but in that case attention is divided between making the proper suction and applying the proper pressure around the glass rod. The use of the seal as a separate affair is the simpler arrangement.

The simplicity and the cheapness of the device described are evident. It can be manufactured in a few moments. The secret of its success lies in the proper adjustment of glass rod and tube but this is quickly learned after a few trials.

A NEW STANDARD FOR THE VAN DEN BERGH TEST

By B. W. RUDD, M.D. and P. H. ADAMS, D.S. ST. WAYNE, IND.

IN MAKING the indirect Van den Bergh test for bilirubin in blood, the ideal standard is an alcoholic or chloroform solution of bilirubin. Since this is not obtainable an ethereal solution of non ammonium alum was suggested, of a strength equivalent to 5 parts per million of bilirubin, as an artificial standard.

There are several objectionable features to this standard:

1 The color of the ethereal iron solution and the azo bilirubin color do not match and the comparison must be for depth of color rather than of matching colors.

2 As the iron standard is an ethereal solution and therefore quite volatile it must be handled carefully and the comparison made quickly, otherwise evaporation of the ether will change the strength of the standard.

3 The standard solution must be made up each time the test is made.

Van den Bergh later suggested¹ an aqueous solution of cobalt sulphate as being stable and satisfactory. While the cobalt standard overcomes the last two objections of the iron standard it still has the objectionable feature that the colors are not identical and that the comparison must be also by depth of color. In our laboratory we have been using with satisfaction, a new

artificial standard, using our regular $\frac{N}{10}$ potassium permanganate as a stock solution. We find that 0.7 cc. of the $\frac{N}{10}$ permanganate diluted to 50 cc. with distilled water, gives a color identical with the azo bilirubin solution.

and is equivalent to 5 parts per million of bilirubin, as compared to the iron standard

Objection may be made that this solution, $\frac{N}{10}$ permanganate, should be standardized frequently. This objection, however, holds also for both the iron and cobalt standards. One of the advantages of the $\frac{N}{10}$ potassium permanganate solution is that it is a standard solution common to all laboratories and must be kept standardized for other tests.

Care must be taken in making $\frac{N}{10}$ permanganate solutions. $\frac{N}{10}$ permanganate solutions should be made and let stand a month to ripen before being standardized. After a month, the top solution should be syphoned off, discarding the last two inches. The syphoned solution may then be standardized in the usual way and will be found to be very stable, no appreciable change occurring under four months. Treadwell and Hall² state their permanganate lost only 1.7 per cent in eight months. Our own solution lost 1.0 per cent in twelve months. This variable is a negligible factor considering the small amounts used in the tests, but may be remedied by restandardization. The permanganate solution should be kept tightly corked, free from reducing vapors and in a dark bottle.

REFERENCES

- ¹McNee and Keefer Brit Med Jour, July 11, 1925
²Treadwell and Hall Analytical Chemistry, 2

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFF M.D. ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

ERYSIPELAS Erysipelas Observations on the Etiology and Treatment with Erysipelas Antistreptococcic Serum Birkhang K. E. Jour Am Med Assn, May 8, 1926, lxxxvi, 1411

In this paper further experimental evidence is advanced to prove that the etiologic agent in erysipelas is a specific type of *Streptococcus hemolyticus* and that the serum produced with this specific type of microorganism possesses very marked curative properties when administered early in the disease.

Clinical trials in sixty moderately severe cases of erysipelas have demonstrated that intramuscular injection of erysipelas antistreptococcic serum in amounts of 100 cc of the unconcentrated and from 15 to 20 cc of the concentrated serum when administered during the first three days of the disease causes a prompt amelioration of toxic depression, a critical fall in temperature and pulse rate, prompt fading of the erysipelatous lesion and rapid absorption of the bluish and edema within the affected areas.

In late cases of erysipelas the result of serum therapy is strikingly favorable in ameliorating the general toxic depression although repeated injections of serum may be necessary completely to neutralize the circulating toxin in the patient's blood. Following each injection of the serum there is noted a critical drop in temperature and pulse to within normal limits as soon as from twelve to eighteen hours after the injection of the serum, the symptoms may quickly return and a second dose may be necessary to bring about a complete recovery.

By means of a skin test dose of toxin injected intradermally and simultaneously with the intramuscular therapeutic dose it is possible to observe whether complete neutralization of the circulating toxins is accomplished or whether an inadequate dose of serum was administered the patient to accomplish the cure of the disease.

It is too early to state whether the serum is purely antitoxic in nature. Its influence on the rate of recurrence of erysipelas in patients having frequent attacks of the disease is also of great interest and will be studied further. It is hoped that annoying attacks of serum sickness following the administration of large doses of antistreptococcic serum will be less severe and fewer when the refined globulin serum is used.

BLOOD CALCIUM Pathologic Variations in the Serum Calcium, Percival G. H. and Steward C. P. Quart Jour Med April 1926 235

Using the method of Kramer and Tisdall for the estimation of calcium in the blood serum the normal range was found to be 94 to 99 mg calcium per 100 cc serum. In four cases, parathyroid administration raised the serum calcium to a level above the normal and maintained it until the drug was withheld. In three further cases the serum calcium was raised by parathyroid administration but fell to subnormal values while the drug was still being given. In two cases parathyroid administration was without effect. In certain cases the effect of the parathyroid was augmented by giving calcium salts. In one case however even combined parathyroid and calcium administration was without effect.

In a case of tetania parathyropriva parathyroid given over a prolonged period failed to influence the very low serum calcium value. When however calcium chloride was given in addition to parathyroid the serum calcium while not reaching the normal level rose considerably.

The administration of calcium salts alone by the mouth failed to produce any increase in the serum calcium in two cases in which the initial value was normal. In two other cases in which a rise was obtained, the initial value was low.

In three different types of endocrine deficiency no effect on the serum calcium which could be directly attributed to the administration of the deficient hormone was noted.

The effect of ketosis on the serum calcium was followed in cases of diabetes mellitus, and in cases of epilepsy in which the condition was induced by dietary measures. In the presence of ketosis the serum calcium was found to be subnormal except during the first days of the condition, when a high calcium was found. No constant relationship between the amount of serum calcium and the occurrence of fits was observed in the case of epileptics.

A table of serum calcium values in various disorders is given. Normal values have been found in chronic ulcerative conditions, while in three cases of lupus erythematosus the value is distinctly subnormal.

PROSTATIC HYPERTROPHY Examination of the Blood in Hypertrophy of the Prostate, Bouet, O., and Eskelund, V. Hospitalstend, Copenhagen, April, 1926, *lvi*, 351.

In the judgment of the authors no great importance can be attached to examination of the blood as a means for the diagnosis of simple hypertrophy of the prostate gland, since in this affection alterations in blood are described as in general too slight in degree to permit of their accurate determination in instances in which figures which lie outside of normal limits are actually encountered.

Material employed by the authors in their search for a positive method of differential diagnosis between cancer and hypertrophy of the prostate gland is declared to have included only doubtful cases of the former, while in eight out of twenty four cases of the latter a condition of hyposinophilia (under 2 per cent) was discovered. In view of the fact that other investigators have noted hyposinophilias below 0.5 per cent, however, the authors regard it as inexpedient to attempt to base a diagnosis of cancer of the prostate gland upon evidence afforded by an hyposinophilic blood picture.

FEVER Distribution of Water and Salts in the Human Organs During Fever, Pribram, E. Arch Path and Lab Med, July, 1926, *ii*, 12.

All living human cells are very rich in water, the distribution of the water in the cell contents being dependent upon the mutual solubility of their constituents which, in turn, depends upon the character of the colloids, and the amount and character of the crystalloids dissolved in the water.

During fever the human organism is in a state of thirst. This state of thirst is caused by a change of the distribution of water in the organism. The parenchymatous organs absorb more water owing to their contents of protein. The causes of the increased demand of water by the parenchymatous organs are of physicochemical nature. The rise of the temperature, the breaking down of proteins and the change of the chemical reaction of the blood (increase of hydroxyl ions) all produce the same effect, an increased faculty of the cell colloids to imbibe water.

Proteins take up more water if the temperature rises from 37° C to 40° C. Organs not so rich in proteins (the skin, the mucous membranes, the subcutaneous tissue) and the muscles lose water, supplying the inner organs with water. The delivery of water by the muscles may be explained by an exchange of their potassium salts for sodium salts. There is an increase of the quantity of potassium phosphate in the urine and a decrease of sodium chloride. The origin of the potassium salts is doubtless the muscle tissue. This tissue has the faculty of imbibing more water in the presence of potassium salts than in the presence of sodium salts. The loss of water by the skin, the mucous membranes and the subcutaneous tissue cannot be explained in a similar manner, but recent articles make it probable that there the nervous (parasympathetic system) exerts a regulating influence.

GLYCOSURIA Nondiabetic Glycosuria John H J Ladoocrinology, March April, 1926, x, No 2, 115

Eight cases of nondiabetic glycosuria have been observed over periods ranging from two to eleven years, during which time the patients have not had diabetes

The patients have had a full diet throughout the periods that they have been under observation

The ages of the patients ranged from two and one half to forty four years at the time the glycosuria was discovered

The literature on nondiabetic glycosuria includes reports of cases for varying periods up to thirty five years, throughout which a normal status was maintained

The highest daily excretion of sugar in the cases reported in the literature was 30 gm per day

Observations of nondiabetic glycosuria show that it is an innocent anomaly requiring no restriction of diet or other treatment

The importance of repeated examinations in cases of glycosuria which is supposed to be nondiabetic in character is emphasized as is the necessity of glucose tolerance tests, in order to make sure that the glycosuria is not due to diabetic or a prediabetic condition

LABORATORY TECHNIC

ALDEHYDES TEST FOR A New Reagent for Ethyllic Propyllic and Allylic Aldehydes Sanchez J A. Semana Med April 1 1926 1, 640

Preparation of reagent The reagent is prepared by dissolving 50 centigrams of piperazine in 10 cc of a 1 per cent solution of nitropotassium of soda. When small amounts of ethyllic, propyllic and allylic aldehydes are added to this reagent a beautiful indigo blue color which is readily diffusible in water but which is somewhat modified by the action of heat is produced

Recognition of ethanal in ordinary ether Ten cc of ether are placed in a test tube, 1 cc of the reagent is added the tube is shaken and if the ether contains traces of ethanal an aqueous stratum of a deep indigo blue color will appear

Recognition of ethyl alcohol in chloroform Twenty drops of sulphuric acid and 0.01 gm dichromate of potash in powdered form are added to 2 or 3 cc of chloroform, the test tube which contains the mixture is shaken a rubber cap which is provided with a flexible rubber tube with two elbows is fitted over the opening of the test tube, the latter is heated, and the vapor is collected in a second test tube which contains 1 or 2 cc water and which is cooled by immersion in water. On addition to the distillate of a few drops of the reagent, if the chloroform contains alcohol, an indigo blue color will be obtained.

Recognition of ethyl alcohol in dilute solution Five cc dilute solution are placed in a flask twenty drops of H₂SO₄ and 5 cc of 5 per cent solution of dichromate of potash are added, the flask is set aside for a period of five minutes, the mixture is then distilled, and the vapor is collected in a tube which has been properly cooled and which contains 1 cc distilled water. When treated with the reagent the fluid which results from condensation will exhibit a deep blue tint which will disappear under the influence of heat

Recognition of the oxyethyl group (HOC) From 5 to 10 centigrams of the substance intended for examination, 5 cc of sulphuric acid (1.1) and 5 cc of 5 per cent solution of dichromate of potash are introduced into a retort, which is set aside for ten minutes after which the mixture is distilled and the vapor is collected in a cooled tube containing 1 cc distilled water. When 1 cc of the reagent is added to a portion of the condensed fluid an indigo blue color which indicates the presence of ethanal is produced

Recognition of lactic acid Lactic acid or lactates are placed in a retort to which a rubber cap and tube similar to those above described are fitted, a few cc sulphuric acid in a dilution of 50 per cent are added, the mixture is distilled until the fluid begins to grow dark, and the vapor is collected in the usual manner. Here the distillate reveals a clear indigo blue tint

HISTOCYTE STAIN Note on the Staining of the Histocytes of the Peritoneum by the Method of Del Rio Hortega, Corria, C, Ramirez, M, and Bianchi, A *Riv Soc Argentina de Biol*, Buenos Aires, December, 1925, 1, 774

In the course of their examination of the origin and function of the different cells of the reticulo endothelial system the authors found it necessary to establish some method of morphologic control of the vital staining realized, and they did this successfully by the ammoniacal silver carbonate method of Rio Hortega To use this method in the peritoneum as in any other class of membranes, subcutaneous cellular tissue, etc, the following rules may be followed

(1) Spread the membrane on a cover glass and cut it so its edges extend a little beyond the cover glass, holding the latter with the fingers by two of its corners (2) Leave it exposed to the air long enough for moderate drying and fix it with 5 per cent formol for an hour at least (3) Cut the membrane in small fragments, it is already firm enough to be handled easily (4) After having washed it in distilled water pass it through the ammoniacal silver carbonate solution prepared according to Rio Hortega's directions for one to several minutes according to the conditions, sometimes a few seconds are enough (5) Reduce it in formol, 1 or 2 per cent (6) Bathe in gold chlorid solution 1 500 (7) Fix in 5 per cent sodium hyposulphite solution (8) Wash and mount

When material is used that has previously been subjected to vital staining the images are superimposed, and when the staining is done rapidly multiple anastomoses of the histocytic elements can be seen when they are in repose and when they enter into macrophagic activity they can be seen developing into round forms In the peritoneum that has not been irritated or subjected to vital staining the classical pictures of clasmatoocytes can be seen clearly in their different morphologic forms, they are particularly abundant in the immediate neighborhood of the blood vessels

To stain the capillary reticular fibers and the intracellular fibrils the same technique used by Rio Hortega for connective tissue can be used, except that the tissue is not fixed so much The steps are

(1) Very brief fixation, two to five hours, cut in frozen sections, (2) Washing in distilled water, (3) Hot silver carbonate until the edges are grayish, (4) Rapid washing in distilled water, (5) Reduction in formol 1 per cent or more, (6) Gold chlorid 1 500, (7) 5 per cent sodium hyposulphite Wash and mount

SPIROCHETES, CULTURE OF Cultural Methods for Increasing the Number of Spirochetes Pallidae in Fresh Syphilitic Tissue, Shaffer, L W *Arch Path and Lab Med*, July, 1926, 11, No 1, 50

Simple aerobic incubation of tissue containing Spirochetes pallida if in blocks of sufficient size, results in a marked increase in the number of spirochetes, as shown in sections stained by the Levaditi method

Their occurrence in almost solid clumps is sometimes most convincing proof of cultural growth in situ These organisms have been shown to be pathogenic when reinoculated into rabbits five days after excision of the tissue

More refined methods of culture (anaerobically in horse serum, etc) offer no advantages over simple aerobic incubation in situ in the original tissue When the tissue is planted in a medium the demonstration of Spirocheta pallida in the surrounding medium affords a rapid method of checking the findings that may be expected in the tissue

Spirochetes were demonstrated in fresh tissue when incubated for periods as long as seventy eight days

The growth of spirochetes inoculated into freshly excised normal tissue does not extend rapidly throughout the tissue but tends to remain localized to the point of inoculation In testicular tissue Spirocheta pallida grows by preference in the interstitial rather than in the parenchymatous portion Renal tissue does not seem to be a suitable medium for its growth

Transplantation of suspected tissue into the abdominal wall of rabbits affords a method of anaerobic incubation which combats the contamination otherwise almost inevitable After an initial increase in the number of organisms, there was a decrease, followed by

their complete disappearance from the tissues by the end of fifteen days. This diminution lasts through the twenty-one day period, but by the end of thirty days the organisms had returned in great numbers. This interval corresponds to the primary incubation period for rabbits. The fate of the spirochetes in the interim is conjectural.

The method of choice for incubation in the application of these findings to the pathologic diagnosis of syphilis remains to be determined. Simple aerobic incubation and transplantation into rabbits seems worthy of trial.

RICKETTSIAE Rickettsiae and Disease Cowdry E. V. Arch. Path. and Lab. Med., July, 1926, 11, No. 1, 59

This paper of twenty-five pages is a comprehensive summary of our present knowledge of rickettsiae and their relation to disease.

A bibliography of seven pages lists all the articles of importance on the subject.

The paper does not lend itself to satisfactory condensation and should be read in the original. Its scope is indicated by the following summary.

Historical introduction

General properties of rickettsiae

Distinction between rickettsiae and cell granules

Criteria for the identification of rickettsiae

Comparison of rickettsiae with the so-called "symbionts"

Distribution of rickettsiae in arthropods

Classification of rickettsiae

The pathogenicity of rickettsiae and reservoir of infection

Status of the different rickettsial species

Relation of rickettsiae to specific diseases

Diseases under suspicion of being caused by rickettsiae

Summary

Bibliography

UREA A Micro Method for Colorimetric Determination of the Presence of Urea in Blood and Urine Cuffi U. Rev. Med. Barcelona March, 1926, 1, 228

Reagents

Trichloroacetic acid 20 per cent.

Nessler's Solution (Folin's formula)

Urease solution 5 gm of newly pulverized soya bean are placed in a mortar and shaken with alcohol for ten minutes, and then filtered.

Standard Urea Solution 0.04 gm pure urea dissolved in 100 cc of distilled water. This solution may be protected with a layer of toluol.

Technic Into a test tube free from ammonia and from mercury (washed with nitric acid and distilled water) are placed 0.3 cc of oxalated blood or of urine (in dilution of 1 to 100) and 0.3 cc of the solution of urease, while 0.3 cc of the solution of urea (0.04 per 100) and 0.3 cc of the solution of urease are introduced into a second tube which has been previously prepared in the manner already indicated. Both tubes are next left for fifteen minutes in the autoclave at 37° C or five minutes in a water bath at 50° C. The tubes are then removed from the autoclave 12 cc of water and 0.6 cc of trichloroacetic acid are added, the contents are well shaken, the tubes are set aside for ten minutes, and the fluid in each is filtered and washed with hydrochloric acid (Berzelius) until a clear filtrate appears. Seven tenths of a cc of the latter are introduced into test tubes which have been washed and dried, and 8 cc of water and 4 cc of Nessler's solution diluted with equal parts of distilled water are added. The colors are now compared with the colorimeter.

Calculation The reading of the standard is multiplied by 0.4 and the product divided by the reading of the unknown. The result represents the milligrams of urea.

For urine the result is multiplied by 100 as it is examined in a 1:100 dilution.

DIABETES Respiratory Quotient Curves in Diagnosis of Diabetes, Petty, O H, and Stoner, W H *Am Jour Med Sc*, June, 1926, *CLXXI*, No 6, 842

The authors believe that if the now generally accepted definition of diabetes mellitus—more than temporarily impaired sugar burning capacity—be accepted, if in a given case, definite impairment of sugar burning capacity be demonstrated by abnormally low fasting respiratory quotient and abnormally small rise in respiratory quotient after a dextrose meal—then diabetes mellitus exists, whether the blood dextrose curve be conventionally diabetic or nondiabetic

Respiratory quotient studies, before, and every half hour for three hours after, the administration by mouth of 1.75 gm of dextrose per kilogram body weight, are a direct index of the normality or abnormality of the sugar burning mechanism

By such respiratory quotient curves absolute differentiation may be made between diabetes mellitus and renal glycosuria, and diabetes mellitus may be diagnosed earlier than by the usual glucose tolerance test

A number of cases, whose blood sugar rose above 180 mg per 100 cc blood and returned to normal in less than three hours, were shown by respiratory quotient curves, to be definitely diabetic

PUERPERAL DISEASES Concerning the Significance of the Changes in the Colloidal Plasma Structures in Septic Puerperal Diseases, Toth, A *Arch f Gynak*, Feb 3, 1926, *CLXXII*, 729

Studies of the albumin globulin fractions thus summarized

Organic bodies in an inflammatory focus split from large molecules into smaller ones. The molar concentration increases, and hypertonemia occurs. The disintegration of the organic bodies produces acid substances, increasing the hydrogen ion concentration in the inflamed region. This process is opposed by another which continually forms the albumin molecules into larger complexes, until finally they are precipitated. The organization of these precipitated masses forms a tissue to replace that whose destruction initiated the whole process.

The picture presented by a septic patient at different stages in the course of the disease depends upon the interaction of the two processes at the given moment. This interaction between injury and defense produces a resultant, the direction of which is determined by numerous factors.

LEPROSY Serologic Analysis of Lepers' Sera, Schöbl, O, and Ramirez, J *Philippine Jour Sc*, March, 1926, *LXX*, 305

Ninety two lepers were examined serologically with the view to deciding certain doubtful points in the serology of leprosy. The question of complement and natural hemolysin content in lepers' sera toward guinea pig, sheep, goat, and rabbit red cells was investigated. The content of hemolytic complement and its keeping quality in the lepers' sera were studied, antishoop and antimoukey immune hemolysin having been used in these tests.

Slight individual differences were found to exist in lepers' sera and in normal human sera alike as to content of natural hemolysins and complement, but no distinct quantitative differences were found between the sera of lepers and those of normal persons. The amount of hemolytic complement in the lepers' sera was found to be the same as that in the sera of nonlepers and is subject to individual variations.

As to the keeping qualities of the natural hemolytic complement, it was found that, in proportion to the original titer, the complement decreased practically at the same rate in the sera of lepers as it did in the sera of normal individuals.

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Marrow was found to give a positive reaction

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EDITORIALS

Sunlight

THE Tuscarora Indians tell us that three thousand years ago the only comfortable place in the universe was the world above the clouds. In this region the clouds themselves were the floor or soil. The atmosphere was always warm and bright and clear, bore indeed every kind of game trooped over the clouds. This happy hunting ground was peopled with the manitous. One a woman, was pregnant with twins and fell by accident through a rift in the clouds. The world below was but a great, cold, dismal swamp inhabited only with turtles, snakes, frogs, beavers and other monsters. Contrary to our traditions, however, these monsters were kindly disposed and when they beheld the goddess descending from the sky, they were greatly concerned for her safety. A great turtle volunteered to receive her on his back and immediately began to expand to broader and broader proportions. Beavers spread mud upon the expanding shell. The mud rapidly increased, becoming thicker and drier until it developed into a great area of soft, rich, fertile country. Bushes and trees grew up in abundance, and by the time

the goddess alighted she was received without injury on a very comfortable couch of moss and earth. The turtle and the soil continued to grow until at the end of a relatively short time they had formed the great continent of North America.

Soon the embryo twin godlings commenced to discuss the most desirable method of accouchement. The more intelligent of the two elected the natural way for his birth. The other, called Badmind, preferred to enter the world by tearing a hole through his mother's side. Each carried out his own plan, but as a consequence of the violence inflicted by the parturient Badmind the goddess died.

The powerful Manitous now existed on the turtle's back, one always doing good, the other always attempting evil. Goodmind, seeing the coldness and dimness of the new earth, took the head from the body of the goddess and sending it skyward transformed it into the sun, the greatest of all spirits. The remainder of the body he converted into the moon that it might illuminate the sky at night. Goodmind, Ni-yoh, the Creator, then populated the earth with birds, fishes, game, animals of all kinds and then placed thereon his *chef d'œuvre*, Man. From this time the Great Manitou was the Sun. The sun, representing the mother of all life on the turtle's back, became the greatest deity of all, and the Sun Dance became one of the most distinctive of the Indian rituals.

Familiarity, however, procures the superiority complex. Joshua assisted materially in dispelling our ideas of the independence of the sun, and the contributions of a multitude of students of the truth have led us to look upon the sun as just another commodity, in a class with oil furnaces, automobiles and airplanes, one which we are glad to have, particularly since there is no tax on sunlight, but which man in his ingenuity could as well do without should the need arise.

But now we learn that the sun is the ultimate source of all life on this earth and that the elements of sunlight in one form or another are prerequisite for the continuance of health in practically all forms of animal and vegetable life. True, we are also informed that man has perfected an apparatus which delivers the active elements of the sun's rays in more efficient concentration than does the sun itself and which produces rays of greatest value which are practically absent from ordinary sunlight as it reaches the earth.

Carbon dioxide, water and inorganic salts were probably the only materials available in the Azoic period from which living matter might have been formed. We are told that the action of sunlight on these appears to have produced formaldehyde, and that radiant energy acting upon this in turn caused the formation of the more complex starches, sugars, amino-acids and even proteins. Organic matter appears to have been built up from inorganic matter by the agency of sunlight acting on water and carbon dioxide in the presence of an inorganic colloid. Life having been initiated, sunlight is necessary for its perpetuation. The green plant will not produce starch unless its chlorophyll is acted upon by sunlight. Chlorophyll is phototropic, changing its position within the cell so as to receive an optimum quantum

of energy Hematin of the blood in its combination with iron is quite analogous to chlorophyll and its associated magnesium Upon exposure to sunlight hematin is said to accumulate in the skin with consequent pigmentation in much the same way as chlorophyll shifts its position under the stimulation of light Also, just as a plant fades and bleaches when it is removed from the light so does the human body become pale and anemic

The substitution of iron for magnesium, which forms a biochemical key note to the metamorphosis from the vegetable to the animal kingdom, was a happy arrangement, for iron is much more readily oxidizable than magnesium and is therefore more satisfactory for the transportation of oxygen from the lungs to the cells of the body Were it not for hemoglobin the average person weighing 150 pounds would require at least 300 pounds of blood plasma to furnish the equivalent quantity of oxygen

Finsen demonstrated many years ago that ultraviolet energy was absorbed directly into the blood through the skin Quinke in 1894 showed that hemoglobin gives off oxygen more quickly in the light than in the dark Both light and heat appear to augment bodily oxidation Pacini claims that day workers transferred to night shifts develop a low grade anemia which may be overcome by exposure to ultraviolet radiation

Malaria relapses in the spring time have been attributed to the action of ultraviolet rays Whitmore produces relapses at will with ultraviolet radiation of malaria infected canaries Quinine has a low grade fluorescence and Bass has observed that the plasmodium may be cultivated in the presence of small quantities of quinine in the dark but that the same amount of quinine in the light will destroy the plasmodium Is light a factor in the destruction of malaria by quinine?

The action of radiant energy on the leucocytes varies with the wave length applied Radium and γ rays decrease the number of circulating lymphocytes Rays in the far ultraviolet produce on the contrary a lymphocytosis Near ultraviolet again tends to lower the lymphocyte count The visible spectrum apparently stimulates both lymphocytes and polynuclears Radiation in the red and infra red zones causes little change The opsonic index is diminished after exposure to γ rays and far ultraviolet Near ultraviolet greatly increases the opsonic effect while visible radiation and infra red produce little effect It seems that in sunlight the near ultraviolet effect predominates Crimer and Drew describe an increase in platelet count following the action of ultraviolet rays Luckiesh and Pacini attribute the speedy convalescence from respiratory infection in part at least to platelet stimulation It has been shown that γ rays and radium will diminish the platelet count more rapidly even than the lymphocyte count, indeed after prolonged radiation the count may drop so low that it never recovers When this happens the animal always dies from an intercurrent infection

"The skin is the place where the external and the internal environment meet for the purpose of reaching an equilibrium compatible with life" Sun burn may be brought on by γ rays or radium or by ultraviolet but not by visible light or by infra red radiation to an appreciable extent

On exposure of the skin to radiation an erythema develops, the more promptly the greater the wave length of radiation. Heat causes a prompt reddening, ultraviolet a slower one and x-rays a delayed erythema. There are two phases or periods of erythema, a primary one consequent on engorgement of the capillaries and a secondary erythema only partially due to engorgement. The latter is followed by melanin pigmentation. The origin of the melanin is not completely understood. Two theories exist, one that it is a degeneration product of hemoglobin and the other that it is a secretion product of pigment forming cells. Luckiesh and Pacini believe that, whether special pigment cells are present or not, the pigmentation seems to be controlled by nerve influence. Cocainized and uncocainized skins exposed to ultraviolet radiation, respectively, did not and did develop pigment. Drugs which activate the sympathetic nervous system appear to promote pigmentation after ultraviolet radiation. These authors suggest that the chromatophores present in the skin of lower animals have in the process of evolution migrated inward and are collected in the adrenal glands and other chromaffin tissues but that these are still intimately associated with the body surface through the sympathetic nervous system. They suggest that pigmentation following exposure is not for the purpose of excluding ultraviolet so much as for promoting its absorption and conversion into utilizable energy. They conceive of pigmentation as a provision whereby the body may continue to benefit from absorption of ultraviolet and at the same time protect itself against the heating effect of the sun's rays. Certain it is that pastes containing melanin protect the skin from the heat of the sun concentrated with a burning glass. The fresh skin contains an oxidase which under the action of ultraviolet light appears to produce a pigmentation just as the freshly cut apple turns brownish on exposure to the air. Oxidase cannot, however, bring about pigmentation except in the presence of phenylalanin, an amino-acid whose constitution is nearly the same as that of adrenalin. Luckiesh and Pacini postulate the interaction of four factors in the production of pigment, namely, ultraviolet radiation, stimulation of the sympathetic nervous system, oxidase in the skin, and adrenalin which serves as an activator.

It has been suggested that ultraviolet radiation striking the skin and accelerating the sympathetic nervous system distributes its stimulus so as to elicit responses from the thyroid, the adrenal, the pituitary and the parathyroid glands.

In some manner, as yet not clearly understood, ultraviolet light affects calcium metabolism. It promotes the healing of bones, and we are told that when hay fever, asthma and colitis are associated with low blood calcium values ultraviolet therapy or ultraviolet therapy combined with calcium administration by mouth produces excellent therapeutic results. Apparently the calcium effect is associated in some way with epinephrin action, and it has been suggested that actinotherapy stimulates the sympathetic nervous system causing an increased secretion of adrenalin which in turn promotes calcium absorption by the tissue cells.

It has long been known that ultraviolet light has a distinct bactericidal activity. The death of the bacterial cell appears to be due to a process of

coagulation. Certain authors suggest that the high concentration of phenyl alanin and tyrosin in the bacterial cell as compared with body cells accounts for this destructive action. These two substances absorb the ray readily. Bacteria in a medium containing them are protected from the destructive action of the ray by the fact that the latter is absorbed by these amino acids in the media.

There appears to be an activation by the actinic ray of foods such as oils, cereals, etc., so that the latter develop greater growth producing abilities, and food so treated is said to develop rickets preventing properties similar to that of cod liver oil.

The above enumeration of the results of ultraviolet action, culled some what at random from the recent monograph by Inclerish and Pacini,¹ presents such an astounding variety of activities and reactions that one is inclined to wonder whether time will give permanent substantiation to the assertions. We have been rather conservative in our selections and one will find many additional remarks and discussions in the volume which are less susceptible to experimental confirmation. As an example we might mention the effect of light on the endocrine system on personality and the like. One is inclined to recall the hyperenthusiasm of some of the early and indeed recent contributions on endocrine physiology.

But if we turn to another recent contribution on sunlight and artificial radiation, one less popular in its exposition and presented as a more formal scientific discussion, coming from the pen of Edgar Mayer of Saranac Lake,² we are again impressed with the undiminished potency of the actinic ray and its effect on many and varied aspects of biology. Among such may be mentioned developmental changes in the embryo, cytotoxic hypertrophy of the parathyroid gland in rabbits irradiated hours laying four times as many eggs as controls, pigmentary changes in hair, increased rapidity of epithelization of granulating wounds, increased endogenous nitrogen metabolism, increased absorption of calcium and phosphorus from the intestine, increased calcium and phosphorus storage, diminution of the blood sugar and many more, and all of this from the action of ultraviolet rays which do not penetrate the skin more than from one tenth to one millimeter.

We shall not attempt to detail the therapeutic indications and uses of the ultraviolet ray. Although recommended in a most diverse classification of diseases, it is no panacea. At the same time we may readily understand how with physiologic responses as diverse as have been described above actinotherapy will have some effect either direct or adjunct in the treatment of many conditions.

The sun is a wonderful phenomenon and the solar spectrum, in all its phases from far ultraviolet to infra red is a marvelous creation. Indeed like the ancients we would gladly again become sun worshipers had we not just read in the morning paper that this celestial orb with its nine thousand degrees of temperature is after all but second rate since we must now compare it with nebulae whose temperatures are measured in millions of degrees.

¹For further discussion of the volumes by Mayer and by Luckh and Pacini see book review section September issue p 11.

were found in both these nerves, but it is possible that they may have come from the vagus through the branches of communication between the nodose and superior cervical sympathetic ganglia "

The superior cervical sympathetic ganglion, among other functions, sends efferent fibers (accelerator) to the heart, and, according to Wiggers,⁸ vasoconstrictor fibers to the coronary arteries and aorta, but apparently does not transmit sensory fibers from the cardiac plexus. This would seem to be borne out in the human by the experiments of Leriche,⁹ since he could produce symptoms like those of an attack of angina by stimulating the first thoracic sympathetic ganglion, and could abolish attacks of angina by anesthetizing the lowest cervical sympathetic ganglion. Stimulation of the superior ganglion produced pain in the jaw and face (Leriche¹⁰) but no indication of reflex is produced by such stimulation if the connection between this ganglion and the nodose ganglion of the vagus is broken (Langley and Jonnesco et Ionescu¹¹). Schittenhelm and Kappis¹² failed to relieve the pain of angina by injection of anesthetic into the upper part of the sympathetic trunk or superior cervical ganglion or the vagus, while injection of the stellate ganglion brought relief. Singer¹³ found that extirpation of the stellate ganglion produced anesthesia of the heart and aorta as far as the left subclavian artery.

From the literature on the surgical treatment of angina pectoris we see that almost every variety of operation from bilateral removal of the cervical and upper thoracic portions of the sympathetic system to the cutting of one small nerve has produced results. The original operation of Jonnesco embodied a principle that has been used by many others since, that is, the operation was designed to cut the afferent fibers from the heart and thereby stop the passage of painful impulses. Coffey and Brown,¹⁴ however, decided on a different approach, and obtained relief from all symptoms of angina by the removal of the superior cervical sympathetic ganglion, which they now think may be due to the cutting of an efferent pathway to the heart. Holmes and Ranson¹⁵ explained the beneficial results of this operation, which they also found effective, on the hypothesis that the pain in angina is due to spasmodic constriction of the coronary arteries, and that removal of the superior cervical ganglion with the superior cardiac nerve "prevents spasmodic vasoconstriction, and, therefore, stops paroxysmal pain, but is without effect on pain caused entirely by structural changes "

The advisability of any operative procedure for the relief of anginal symptoms should be based on the location of the pathology and the origin of the symptoms. As has been pointed out, the solution to this problem is yet to be found. The pathology has been located in the myocardium (Mackenzie¹⁶), in the aorta (Albutt), and the coronaries. Head's¹⁷ studies on referred pain have demonstrated that pain referred from the aorta has a distribution over cervical segments 3 and 4, and thoracic segments 1, 2, 3 (and 4?), the peripheral area which is affected in angina pectoris, while pain referred from the heart valves or myocardium shows its effects in segments for the most part lower in the thorax. As the coronary plexus arises from the aortic plexus embryologically, pain from the coronaries could not be distinguished from that arising in the aorta. This would seem to indicate that whatever pathology

of angina pectoris, the pain stimulus originates in the aorta or coronaries. Senger could produce pain by stimulating the adventitia of the coronaries or the aorta, the latter being especially sensitive to stretching, and pain from the epicardium and pericardium, but could not produce pain by stimulation of the heart muscle or endocardium by mechanical, chemical, or electrical methods. He also observed that an increase in blood pressure caused dilatation of the bulb of the aorta, a fact which may be significant in angina. Head¹⁸ observes that tension is the most effective stimulus of any hollow organ, and Wenckebach states that whatever stimulates the action of the heart, increases its output or raises the blood pressure causes the pain" in cases of angina pectoris.

Nathanson¹¹ emphasizes that coronary disease may or may not accompany angina and vice versa. Though there has been no demonstration of a definite pathology of the coronaries which is always associated with angina pectoris, this does not preclude the possibility that the pain stimulus arises from them. It should be evident that the pathologic cause of the anginal pain is not necessarily acting at the point of origin of the pain. For example if the pain is due to spasmodic contraction of the coronaries the pain stimulus arises in the coronary walls but the cause of the spasm could be an irritative lesion in the superior cervical sympathetic ganglion, the central nervous system, the stellate ganglion, or in the pathways between these points, or some factor acting on the sensory endings in the heart or vessels. Danielopolu⁶ credits a physiologic explanation of the pain as due to inadequate coronary circulation, and its effects on the heart wall but supposes a vicious circle of reflex stimulation originating in fatigue toxins in the heart to account for the phenomenon. Penfield¹ suggests a theory of reflex pain supposing an arc reflex from the heart which would cause vasoconstriction throughout the area supplied with efferent fibers from one or more sympathetic ganglia, and the peripheral vasoconstriction would then stimulate the somatic end organs for pain.

There has been little attention paid to the study of the ganglia removed from anginal patients. Ormos describes the changes in the sympathetic ganglia from three fatal cases of angina finding pigmentation of the nerve cells, degeneration of some nerve cells and fibers, an increase in the connective tissue around the blood vessels and nerve fibers and some lymphocytic infiltration. One of these patients was 78 years old, another 72, and the third, though the age was not given, had suffered from angina for nine years. Ormos claims that the degeneration of the ganglion cells is primary and is followed by changes in the coronaries. Since then Shave³ in well controlled experiments, found that experimental irritation of the vagus, the superior cervical sympathetic nerve, or the depressor nerve in the rabbit will result in endarteritis of the aorta, more particularly of the first part of the aortic arch. It is conceivable that the beginning of such changes even before they were demonstrable microscopically might stimulate pain fibers in the vessels, or cause spasmodic contraction which would stimulate pain fibers, or account for insufficient coronary circulation.

Staemmler²⁴ examined ganglia removed at operation from cases of vasomotor neuroses, and three cases of angina pectoris. The patients were respectively 60, 52, and 56 years old. He reports that in the ganglia from two of the patients a majority of the cells were normal, but some were swollen and had lost nuclei, and that the total number was decreased. In addition, the connective tissue was increased and there was some lymphocytic infiltration. The ganglia from the third case were negative. He further points out that the same changes were found in ganglia from cases of Raynaud's disease.

Obendorfer²⁵ stated that the changes described by Staemmler were not specific for angina pectoris and were not different from the appearance of ganglia from old individuals. Terplan²⁶ examined the sympathetic ganglia from a great many cases of a variety of diseases, acute and chronic, and agrees with Obendorfer that there are no specific changes in ganglia from anginal patients and that changes described come within the limit of normal variation.

The ganglia we have studied are from six cases operated upon by Dr. Graham at Barnes Hospital and an additional one (Case 7) sent in from outside. While the descriptions are based on the superior cervical sympathetic ganglia, it must be stated that more than that ganglion was removed. In most cases as much of the cervical sympathetic trunk and superior cardiac nerve were excised as could be reached. This included the middle cervical ganglion in some cases, and, in one case in which an atypical ganglion was found, the trunk was removed to a point below the clavicle. It is evident, therefore, that in some cases sensory fibers from the cardiac plexus were removed as well as the efferent group from the superior cervical ganglion. A brief account of the cases follows.

CASE 1—Male, age sixty seven, for about a year had had attacks of rather typical anginal pain beginning over the heart and radiating to the left arm. At operation a large atypical ganglion described as extending from the mastoid process to below the clavicle was removed from the left side. Until the time of his discharge from the hospital, he was free from further attacks. Eight months later he was readmitted with a carcinoma of the rectum. He died from bronchopneumonia after the establishment of a colostomy. He had been free from further anginal attacks since the sympathectomy.

CASE 2—Male, age fifty eight, for six months had attacks of substernal pain which radiated into both arms, hands, and up into neck and face. The pain was most marked on the left side. At operation the left superior and middle cervical sympathetic ganglia and intervening portion of the trunk were removed. After a short period the pains returned, though they were said not to be so severe. In addition a buzzing of the ears came on and persisted. When seen one year later, the patient stated that he was much improved and had had only a few slight attacks of cardiac pain. He stated also that he was glad that he had had the operation.

CASE 3—Male, age fifty one. For six months the patient had severe substernal pain. This was a case of aortitis, and removal of the left superior ganglion and trunk did not permanently relieve the symptoms, as they returned on the third day following the operation. A laminectomy was performed later and dorsal roots 1, 2, 3 and 4 were cut. This brought relief, but the pains returned on the right side and the patient gradually failed and died two months later. Autopsy showed an advanced syphilitic aortitis and a generalized arterio-sclerosis, including the coronaries. The sympathectomy was performed on this patient largely as an experimental procedure, the patient having complete understanding, because of the intractable pain.

CASE 4—Male, age sixty-five. For about two years the patient had suffered from attacks of pain which radiated into both shoulders and which was relieved by amyl nitrite. The pain finally spread into both arms and hands, but there was no feeling of suffocation or pressure, and no pain over the heart or subternally. The right superior cervical sympathetic ganglion and part of the trunk were removed resulting in relief for the patient.

CASE 5—Male, age forty-eight. For more than two years the patient had typical attacks of anginal pain gradually increasing in severity. The left superior cervical sympathetic ganglion, the superior cardiac nerve and some of the trunk were removed at operation. The pain was relieved except for a very slight pain in the left arm which was not severe enough to bother the patient. When seen eighteen months later the patient had been carrying on his normal office work for more than a year without discomfort and had also married.

CASE 6—Male, age sixty-one. Had suffered from anginal attacks for about five months. The left superior cervical sympathetic ganglion, the superior cardiac nerve and several centimeters of the trunk were removed but the patient died suddenly a few hours later while sitting up in bed talking to his wife.

CASE 7—We have unfortunately only the ganglion and trunk and no history.

An analysis of the cases shows there was one (Case 3) of syphilitic aortitis, and five more or less typical cases of the chronic form of angina pectoris. Of these five, one (Case 6) died after the operation and before it was possible to determine its effectiveness and four experienced various degrees of relief. Case 1 was relieved but since in this one was found the atypical ganglion which was removed with the trunk to a point below the clavicle, it is possible the relief obtained could be due to destruction of sensory pathways from the cardiac plexus. Case 2 experienced some relief but the pain was originally bilateral and has persisted in part in a rather severe form. In this case sensory pathways were undoubtedly destroyed in the removal of the middle cervical ganglion. Case 4, in which the pain was not substernal but in the shoulders and arms was relieved by a right cervical sympathectomy. Case 5 had the typical anginal attacks and great relief followed the operation only a slight endurable pain occurring at times in the left arm but in this case as also in Case 7 more than just the superior cervical ganglion was removed and sensory pathways were possibly cut. The operations performed on the above cases differed therefore from those of Coffey and Brown in which only the superior cervical ganglion was removed.

In the study of the ganglia from the above cases we have attempted to observe the size and shape of the nerve cells, the amount of pigment, the state of the Nissl material, the relative number of capsule cells, the amount of connective tissue, the condition of the blood vessels and the presence of leucocytes. The ganglia from operated cases were compared with superior cervical sympathetic ganglia from normal dogs and from autopsies of three patients who had not had angina. One of these patients was a boy ten years old who had suffered from rheumatic fever. The ganglion showed one or two small areas of lymphocytic infiltration but was otherwise normal and well preserved. Terplan showed that of the ganglia from 28 cases of acute infectious disease, twenty showed no changes at all and the changes in all but two of the remaining eight could be considered negligible. Another of the ganglia from autopsies was that of a man aged 63 who had a tumor of the thyroid which obstructed the esophagus and who died in a state of extreme emacia.

tion, weighing less than 70 pounds. Though there was an apparent increase in the pigment of the cells of this ganglion it was otherwise normal in appearance.

Method—Parts of ganglia and intact ones were prepared by the pyridin-silver method. Others were fixed in Bouin's or Zenker's solution, and others in 5 per cent acetic acid in 95 per cent alcohol. Following Zenker's or Bouin's solution sections were stained with hematoxylin and eosin. After the acetic alcohol mixture some sections were stained with toluidin blue for Nissl granules and others with hematoxylin eosin. In addition to these methods, portions of the sympathetic trunk were stained with osmic acid in three cases and yielded interesting results.

The results of the histologic study of the sections of the seven superior cervical sympathetic ganglia and the one middle cervical ganglion from Case 2, stained by the pyridin-silver method, with toluidin blue, and with hematoxylin and eosin, were essentially negative so far as a specific pathology associated with angina pectoris is concerned. It is true there was considerable brownish pigment in the cells of some ganglia, especially in those from older individuals, in which a majority of the cells were pigmented. This pigment stained black with osmic acid. There was also pigment in numerous cells in the ganglia of a ten-year-old boy who died of rheumatic fever, and Terplan, et al, have pointed out that pigment increases with age. There were occasional examples of lymphocytic infiltration in small areas of the connective tissue of Cases 2 and 3, though this was not found in other cases, and was found in ganglia from nonanginal patients. The Nissl material in a few cells of each ganglion showed a chromatolytic change, but this occurs in normal ganglia of both sympathetic and cerebrospinal type. Characteristically the Nissl material of the cells of the superior cervical sympathetic ganglion is arranged in a peripheral ring with only a few cells showing scattered granules in the rest of the cytoplasm. This point distinguishes the ganglion in section from the nodose ganglion of the vagus with which it is intimately bound, since the cells of the latter contain medium sized Nissl granules evenly scattered through the cytoplasm. There was no increase in the connective tissue of the ganglia, nor any apparent change in the number or size of cells or fibers as compared with control ganglia. The blood vessels did not show any evident arteriosclerotic change, though they were congested in a few instances, no doubt as the result of the operative procedure.

One of the most interesting findings in the series was in the osmic acid preparations. A portion of the cervical sympathetic trunk just below the superior cervical ganglion from each of three cases was stained in osmic acid and studied for myelinated fibers. In such a section could be made out different fascicles which could be identified according to their content of nerve fibers. As described by Ranson and Billingsley this portion of the sympathetic trunk shows a field of myelinated fibers of uniform small size (Fig 1 C). They are preganglionic fibers passing to the superior cervical ganglion. Associated with the actual sympathetic trunk are nerve bundles which contain postganglionic fibers such as make up the superior cardiac nerve, for example, which course with the trunk for a short distance. These bundles of postganglionic

fibers are for the most part unmyelinated (Fig 1 A), though a very few are of the small myelinated variety. In each of the osmic acid preparations we observed small clumps of large myelinated fibers (Fig 1 B) which resembled those sensory fibers traced by Lidgeworth, Ranson and Shawe from the cardiac plexus through the lower sympathetic connections. They measured from 10 to 16 micra in diameter and were therefore distinctly larger than the small myelinated fibers of the sympathetic trunk which averaged about 4 micra in diameter. There were two or three clumps of these large fibers with an occasional solitary one the total number being thirty or more in one section. The majority of these large fibers occurred in fascicles of postganglionic unmyelinated fibers and the occasional ones which were mingled with the preganglionic group were rather near the edge where there were small clumps of unmyelinated postganglionic fibers.



Fig 1—Sympathetic trunk just below the superior cervical ganglion. Osmic acid $\times 100$.
 A. Unmyelinated (effluent) bundle. B. Clump of large myelinated fibers. C. Small myelinated fibers of the sympathetic trunk proper.

Concerning the origin and course of these large myelinated fibers we can at present only speculate. One series of preparations included a section of the sympathetic trunk below the point where the superior cardiac nerve separates from it and the clumps of large fibers were absent at this level. Higher in this series the fibers were present in a section which included the lower pole of the superior cervical sympathetic chain in connection with the superior cardiac nerve or some small nerve nearby. Because of the size of the fibers it would hardly be reasonable to suppose that they were postganglionic fibers from the superior ganglion. They are most probably sensory fibers and if so, probably come from some cerebrospinal nerve. Langley states that the vagus sends half a dozen to a dozen nerve fibers to the superior cervical sympathetic ganglion and that a few myelinated fibers from the glossopharyngeal nerve sometimes enter the head end of the ganglion. Experimental reflexes through the vagus from stimulation of the superior cervical sympathetic

ganglion have been demonstrated by Langley, Jonnesco and Ionescu. The latter authors² also suggest a possible sensory pathway from the heart through the vagus to the superior cervical sympathetic ganglion and then to the cord by way of the rami communicantes of the upper cervical nerves. Langley observes that in the cat the depressor nerve contains "an appreciable number of sensory (pain) fibers" and suggests that this may be so in man. It is not beyond the range of possibility that these large fibers we have observed belong to the much disputed depressor nerve. As stated above, Ranson observed a few large myelinated fibers in the cervical sympathetic trunk and the superior cardiac nerve from at least one patient. Further investigation may show that these fibers are of more constant occurrence than has been supposed.

SUMMARY AND CONCLUSIONS

From the histologic study of the ganglia from the above seven cases, we can safely say that no changes specific for angina pectoris have yet been demonstrated in the superior cervical sympathetic ganglia. Though there may not be changes in the ganglia recognizable under the microscope, the pathology may be located in the superior cervical ganglion. The relief from anginal pain obtained by removal of the ganglion has not yet been given a satisfactory explanation. The idea that it is due to severing the vasomotor pathway to the coronaries and aorta is as yet only a hypothesis. There has been no conclusive proof given of the existence of such a vasomotor pathway. Relief obtained from operations which are different from that of Coffey and Brown in that more than the superior cervical sympathetic ganglion is removed might be explained as due to interference of known sensory pathways from the heart which exist in the lower portions of the cervical sympathetic trunk.

Clumps of large myelinated fibers resembling the sensory fibers of other parts of the sympathetic nervous system were present in the postganglionic bundles bound with the cervical sympathetic trunk at the inferior pole of the superior ganglion in the three cases examined. The origin and course of these fibers is not known.

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HYPOGLYCEMIA*

WITH AND WITHOUT INSULIN WITH AND WITHOUT SYMPTOMS

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WE DESIRE to report on three patients recently observed with low blood sugars, two with the symptoms usually associated with hypoglycemia, and induced by insulin, the other patient free of such symptoms and never having had insulin †

CASE 1—R. S., woman, aged 65, diabetes mellitus complicated by gangrene of the left foot

She was receiving insulin, five units three times a day while on a diet containing 60 gm of carbohydrate, 45 gm of protein, and 100 gm of fat a total of 1407 calories. The blood sugar values were distinctly above normal (Table I) when suddenly, while on the same diet and treatment, the patient became comatose, developed a right facial paralysis and left hemiplegia, and stertorous respiration. The blood sugar at this time was 0.02 per cent (27 mg per 100 cc.) Insulin was discontinued and glucose forced by mouth and intravenously, 50 gm of glucose were administered by stomach tube and 200 cc of

TABLE I
CASE 1

DATE	BLOOD SUGAR PER CENT	INSULIN UNITS PER		DIET			TOTAL CALORIES	REMARKS
		DOSE	DAY	CH GM	PROT GM	FAT GM		
10/5/26	0.133							
11	0.192	5	15	60	45	105	1407	Urine Acid 2.4 Blood Urea N 12.0 Chemistry
12	0.129	5	15	60	45	105	1407	
13	0.194	5	15	60	45	105	1407	
15	0.126	5	15	60	45	105	1407	
16	0.141	5	15	60	45	105	1407	
18	0.027	0	0					Sudden Coma and Hemiplegia Relieved by glucose by gavage and intravenously
19	0.223	0	0	60	45	105	1407	
21	0.174	0	0	60	45	105	1407	

From the Department of Medicine New York Post Graduate Medical School and Hospital

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†The blood chemistry determinations in Cases 1, 2, and 3 were carried out by the Department of Laboratories New York Post Graduate Medical School and Hospital

TABLE II
CASE 2

DATE	BLOOD SUGAR PER CENT	B P	BLOOD UREA N, MG PER 100 CC	BLOOD CO ₂ CP	DIET	TWITCH INOS	VOMIT-ING	NAUSEA	GLUCOSE		REMARKS
									INTRA VENOUS	MURPHY DRIP	
9/25/26						BEGAN	0	BEGAN			Preceding admission to hospital
26						++	0	++			
27						+++	0	+++			
28	0.063	224/140	76.8	54.8	High starch	+++	0	+++		400 cc 5% Glucose	
29		198/138			" "	+++	+	++			
30	0.030	204/140			CH gm 150	+	+	++	25 G		
10/1/26	0.094	204/136			CH gm 300	+	+	+++	65 G		
2		194/122			High starch	+	++	++	75 G		
3		190/110			" "	+	++	+++		350 cc 10% Glucose	
4	0.0750			59.8	" "	+	+	++	50 G	225 cc 10% Glucose	
5		182/118			" "	+	0	++			
6					" "	+	++	+++			
7	0.116	202/124	85.4		" "	+	+	+			
8					Very little food eaten		++	+++			
9		184/110			" "	+	+	+++			
10	0.108	182/110			" "	+	+	+++			
11	0.107			51.3	" "	++	++	+++			
12					" "	+	++	+++			
13		180/116			" "	+	++	+++			
14					" "	+	+	+			
15					" "		++	+++			Murphy Drip (K Acetate 1 dram)
16		188/114			" "	0	++	+++			(H ₂ O 500 cc)
17		148/92			" "	0	+++	+++			(H ₂ O 500 cc)
18	0.120	166/86	123.6		" "	0	++	+++			From 10/16 to 10/30 received chloral 40 gm daily in 8 oz glucose solution per rectum
11/2/26					" "	+	+++	+++			

Died in uremic coma 8:45 P M

25 per cent glucose solution intravenously. The patient became conscious during the infusion and the palsy disappeared. The next day the blood sugar was 0.223 per cent, and three days later, 0.174 per cent. She left the hospital improved.

CASE 3—A S male aged 38 chronic nephritis with anemia and renal insufficiency uremia resulting in death. The patient had scarlet fever as a child. He accidentally discovered albumin and casts in his urine while a medical student sixteen years ago. Since then a chronic diffuse nephritis with progressive hypertension, anemia and renal insufficiency has developed. He was admitted to the New York Post Graduate Hospital on September 23, 1926, with a history of nausea, vomiting, vertigo, headache and muscular twitchings of arms and legs for four days preceding admission. The blood pressure was 224/140, the blood urea nitrogen 76.8 mg per 100 cc, the CO combining power of the blood, 54.8 volume per cent and the blood sugar 0.063 per cent. Treatment was instituted to combat the uremia. The patient received a high starch diet and a Murphy Drip of 400 cc of 5 per cent glucose solution was given per rectum.

The blood sugar taken two days later was 0.030 per cent (30 mg per 100 cc). This determination was checked on another blood specimen and found to be correct. The patient was conscious and mentally normal. The twitchings and nausea and vomiting continued, the tremors, however, being less frequent and less violent than on admission despite the very low blood sugar. The further observation that the twitching and nausea were present when, after intravenous glucose injections the blood sugar was 0.750 per cent (750 mg per 100 cc) leads us to conclude that these symptoms were not those of hypoglycemia, but of uremia which finally caused the death of the patient. Despite progressive nausea and vomiting and the increasing development of uremic coma the blood sugar later never dropped below a normal level. It must be remembered that this patient never used insulin. See Table II.

CASE 3—Mrs L, a woman aged 60 diabetes mellitus chronic interstitial nephritis hypertension, blood sugar was zero during a hypoglycemic reaction. Despite the seriousness of her case she did not cooperate with her physicians. She did not adhere to her diet and frequently omitted her insulin injections making it very difficult to keep her under control. On Oct. 27, 1926, her blood sugar was 0.260 per cent and CO combining power 27 volume per cent. She received 10 units of insulin at 9 A.M. and at 1 P.M. 15 units of insulin. At this time 120 cc of orange juice were given by mouth. At 4 P.M. the procedure followed at 1 P.M. was repeated. Her symptoms (headache, nausea, flushed cheeks and coated tongue) improved. Two and one half hours after the last insulin injection (see Table III) the patient was bathed in a profuse cold sweat, she was semicomatose, morning and irrational with jerking movements of the extremities. The temperature was subnormal and the pulse weak, thready at a rate of 136 to 140 per minute. She had vomited a greenish

TABLE III

CASE 3

DATE	TIME	BLOOD SUGAR %	BLOOD CO ₂ % VOL	INSULIN UNITS	ORANGE JUICE	SYMPTOMS	REMARKS
10/26/26	Evening	-	-	10	-	Headache flushed cheeks nausea dry skin coated tongue	
10/27/26	9 00 A.M.	0.260	27	10	-		
	1 00 P.M.	-	-	15	120 cc		
	4 00 P.M.	-	-	15	120 cc		
	6 30 P.M.	0.000	-	-	-		
	9 00 P.M.	-	-	-	-	Profuse sweat semicomatose, irrational jerking extremities feeble pulse, rate 136 Improved after atropine adrenalin digitalin orange juice	
10/28/26	9 00 A.M.	0.045	50	-	-	Rotational feeling well	

fluid and a little orange juice. The blood sugar at this time was zero (as determined by the biochemist S. Senn). There was absolutely no reduction of the alkaline copper solution by the Folin-Wu method. The test was repeated with the same result. The patient improved after having 1 cc of adrenalin chloride solution and 1/150 grain of atropine sulphate by hypodermic injection followed by forty drops of digalen. She was soon able to retain small amounts of orange juice by mouth. At 9:00 P.M. of the same evening she was rational and in fairly good condition. The blood sugar at 9:00 A.M., the following morning (Oct 28) was 0.045 per cent and the CO combining power 50 volume per cent. The patient was perfectly rational and felt very well. Table III summarizes the data on her case.

A case similar to our third, is that reported by Millard Smith,¹ of a boy of four years, in whom, at the height of an insulin reaction, the blood was found to contain no sugar at all by the latest Folin-Wu method for blood sugar. The boy had been given his breakfast and twelve units of insulin. One hour later he was sleepy. When awakened three hours after the insulin he was drowsy, irritable, his eyes were drawn to one side, his skin was moist, pale and cyanotic, and his pulse rate 135. He vomited his breakfast and some orange juice and the observation is recorded that his color and stupor improved after vomiting. As the author of this report states regarding the boy's blood, "there was not the slightest reduction of the alkaline-copper-tartrate solution during the boiling, nor of the phosphate molybdate reagent when added. The blood sugar was zero. The determination was later repeated with the same result." The boy recovered and no further hypoglycemic symptoms were noted.

The comment of Stanley R. Benedict on an experimental result is worth recording. In a dog with convulsions following insulin injection, the blood sugar was 27 mg per 100 cc by the Folin-Wu method (old) and 16 mg by Benedict's alkaline-copper method. Stating that even the figure 16 mg was too high, Benedict regarded the blood of the dog "as free from glucose."

SYMPTOMS AND SIGNS

The following is a summary of the accepted symptoms and signs of hypoglycemia as reported by the Toronto school and others.^{3, 4} The patient usually first became aware of hunger, or a sense of fatigue and weakness at a blood-sugar level of about 0.07 per cent. It was noted that, while the extent of the fall of the blood sugar was somewhat dependent upon the initial blood-sugar level as well as the dosage of insulin, the intensity of the reaction to any given quantity of insulin could not be predicated accurately. The hunger was followed by anxiety, nervousness or the so-called "inward trembling," though, usually, no actual visible tremors were noted. This feeling of apprehension was sometimes accompanied by loss of emotional control in which the patient became excited or experienced crying spells, a lack of coordination of fine movements was noted as well as aphasia, confusion, disorientation and delirium, vasomotor phenomena were frequent, pallor or flushing, at times alternating dilated pupils, a sense of heat or chilliness, a rapid pulse which was thought to be especially true in children, almost always a profuse perspiration, as characteristic a symptom of the later stages of the reaction as hunger and weakness were of the earlier. When the blood-sugar level

reached 0.03 per cent, the patient was usually in coma, with hypotonia, loss of deep reflexes, low temperature, and convulsions at times terminating fatally. As inconstant signs, deafness and difficulty in articulation were noted.

We believe that the cases whose histories have been cited, as well as the review of the literature, demonstrate that the signs and symptoms of hypoglycemic reaction are subject to wide variations. It is particularly important to realize this, as serious consequences for the patient may arise if this is not taken into account. The suppression of conscious control and the substitution of automatic activity is especially embarrassing and dangerous. Thus we have one patient, a young woman of twenty years, who has been treated for diabetes for eight years. She now requires small doses of insulin, five or six units twice a day, this dosage in the normal individual is usually not productive of any reactions. In her case, however, if a meal is delayed too long, or for no accountable reason, she becomes an automaton, voluntary control of thoughts and actions, and memory are absolutely lost for the time being. One evening she administered her insulin and boarded a taxicab, subsequently she has no recollection of her actions until she was seated at a large dinner table, the banquet half finished and many of the guests astonished and amused at her behavior and conversation. In other cases, patients have wandered about a theater during the performance, have stood in the middle of traffic in a crowded street, have become violent attempting to break up furniture and resisting interference, and have exhibited various manifestations that make it evident that in certain individuals especial supervision must be considered, for the same type of symptom is prone to recur in each person. It is not desired to convey the impression that these occurrences are common—in fact they are rare—but they must be taken into account as possibilities and due allowance made for them, and proper measures instituted with such patients. Sometimes these episodes are amusing, at others dangerous to the individual and under certain circumstances as in the case of men of national prominence may become a menace when policies, speeches and actions are threatening or indelicious.

BLOOD SUGAR LEVEL

We have observed several cases in whom the hypoglycemic reactions occurred while the blood sugar was at a normal or even higher than normal level. One of these we were fortunately able to follow through such a reaction, the details are given in Table IV.

TABLE IV

MODERATE HYPOGLYCEMIC REACTION AT A BLOOD SUGAR LEVEL OF 0.075 PER CENT AND HIGHER IN A CASE OF DIABETES MELLITUS PATIENT AGED 40 YEARS

TIME	INSULIN UNITS	BLOOD SUGAR PER CENT	REMARKS AND SYMPTOMS
9 00 A M	10	0.183	Fasting throughout
9 00 A M			
11 00 A M		0.075	Weakness pallor perspiration tremor of hands
1 00 P M		0.083	Same symptoms but less marked
3 00 P M		0.111	Comfortable but still weak

Considering the cases reported in Tables II and IV, it can be definitely concluded that hypoglycemic reactions *may* occur at normal blood-sugar levels (Table IV) and may remain absent even when the glycemia is markedly depressed (Table II) (as low as 0.030 per cent, in the present instance)

Children apparently tolerate hypoglycemia much more readily than adults. We have had one case in a boy, aged twelve, in whom a reduction of the blood sugar by insulin from 0.326 per cent to 0.052 per cent in three hours resulted in *no* hypoglycemic manifestations. Dr. Lewis Frissel mentioned a similar instance to one of us in which the blood sugar dropped to about 0.040 per cent. The blood sugar in children is generally lower than in adults. The average figure observed by Meitz and Rominger,⁵ four hours after the children's meal, was 0.081 per cent in healthy infants, irrespective of age and mode of feeding. It is perhaps because of the low blood sugar characteristic of normal children that they may have a very low sugar level with few or no symptoms after insulin administration. Thus Genevieve Stearns⁶ records the figures in two diabetic children, after insulin, of 0.036 per cent, 0.042 per cent and 0.025 per cent with no symptoms at all at first, and at 0.025 per cent drowsiness, flushed appearance, free perspiration and inability to walk alone, though the child was conscious. In the second child, the only symptom was drowsiness with blood-sugar determinations reading 0.030 per cent, 0.033 per cent and 0.034 per cent.

It becomes apparent that in adults hypoglycemic reactions may occur at fairly high blood-sugar levels and not necessarily with low concentrations, in children, usually, the percentage of glucose in the blood must be very much depressed before symptoms become manifest.

While the subject of *hyperglycemia* has been extensively investigated both before and since the discovery of insulin, the study of *hypoglycemia* is more recent and closely associated with the discovery of insulin, though Fischler,⁷ in 1913, described the weakness and convulsions produced in rabbits while lowering their blood sugar by fasting, phloridzin, or epinephrin. He also reported the recovery of the animals after infusions of glucose and termed their previous condition "glycopraeval intoxication."

INANITION AS A CAUSE OF HYPOGLYCEMIA

Inanition is one of the earliest known causes for hypoglycemia. Abstinence from food, especially when coupled with excessive consumption of the body's storage of glycogen and glucose (as occurs in hard exercise and vomiting), appears to be one of the common reasons for unexpected and even marked lowering of blood sugar. Case 2, reported above, may come in this category, in this instance the vomiting may have been a contributory factor in lowering the blood sugar to 0.030 per cent. Guy⁸ observing children, noted a distinctly lower blood sugar after vomiting. Joslin⁹ reports a nondiabetic with prolonged undernutrition produced by partial pyloric obstruction present over a period of years. This patient, three hours after a meal, had a blood sugar of 0.05 per cent.

In treating epileptic children by fasting, Talbot, Shaw and Moriarty¹⁰ observed that the blood-sugar readings fell remarkably, as for example, to

38 mg per 100 cc in one child, who, as a result, was frightened and nervous, experienced oppression and pain in the chest and throat, and vomited. Another child's blood sugar level fell to 48 mg per 100 cc. All the children were relieved by feeding glucose. In spite of the definitely low blood sugar, clinical symptoms were absent in most of the children.

The investigation of the runners in the Boston Marathon Race by Levine, Gordon and Derick¹¹ in 1924 and again in 1925 furnishes an example of the effect of extreme physical effort. Blood sugar figures as low as 0.045 per cent were obtained, and the symptoms of exhaustion when analyzed closely resembled those of 'insulin shock'. The addition of sugar to the diet of the same participants in the race of the succeeding year appeared to ameliorate some of these symptoms.

The inability of the body to absorb glucose (fasting in another aspect), may also have some bearing on the production of hypoglycemia. Mertz and Rominger³ record that the absorption of dextrose is least in "dried out" infants. This may explain the observations of Guy⁸ that whereas the normal sugar levels were 0.060 per cent to 0.090 per cent those of 'atrophic' infants, three to four hours after meals went as low as 0.037 per cent. Low blood figures were reported by Chipin and Myers¹² in 'atrophic intoxicated' children.

Lack of water content in the body may have been one of the factors in bringing about hypoglycemia in the cases just mentioned, indirectly, it may produce inanition. The following cases in adults tend to confirm the idea that loss of fluid in the body may be responsible for such results. In a case of dehydrating diarrhea observed by Lin and Chang Hsio¹⁴ in China a blood sugar of 0.075 per cent was obtained with carpopedal spasm, rigidity of the abdominal muscles, tremor of the body, pallor, profuse cold perspiration, weak and rapid pulse, numb and stiff face and extremities. The authors question whether or not the cramps occurring in cholera are not due to a low blood sugar. In diabetic inanition Joshi¹⁵ reports a case complicated by diarrhea and tapeworm where the blood sugar fell to as low as 0.017 per cent shortly before death. In children a compensatory effect of water and glucose has been noted in the fasting children with low blood sugars studied by Talbot, Shaw and Moriarty¹⁰ so long as large amounts of water were ingested and excreted there were but few subjective symptoms, whereas the material diminution of water intake induced flushing, anxiety, languor and abdominal pain—all capable of relief by glucose ingestion.

SUMMARY

1 The cases reported in this paper demonstrate that hypoglycemia may develop with marked suddenness both in diabetics and nondiabetics, in patients receiving comparatively small doses of insulin and those who have never had insulin.

2 The symptoms accompanying the depression of the blood sugar vary a great deal, they may resemble those characteristic of other diseases and lead to incorrect diagnoses as, for instance, the hemiplegia of our first case. Especial attention is called to the serious situations created when hypo

glycemia, brought on by insulin administration, results in loss of voluntary control with resultant irresponsible actions

3 There may be no symptoms though the blood sugar be very low (0.30 per cent, in one of our adult cases), such instances apparently are not very rare in children, but occur infrequently in adults. On the other hand, in certain individuals hypoglycemic reactions manifest themselves, though the blood sugar be at the accepted normal levels (0.75 to 0.083 per cent in a reported case)

4 One diabetic patient is reported whose blood sugar after moderate insulin therapy (45 units in twenty-four hours) was at the zero level

5 Inanition and dehydration are discussed in their relation to the production of hypoglycemia, these factors may have been responsible for the low blood sugar found in one of our cases

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THE RELATION BETWEEN CARDIAC REACTIONS TO DRUGS AND THE P_H OF THE BLOOD 2 EXPERIMENTS WITH MERCURY*

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AS STATED in a recent communication¹ from this laboratory, changes in the P_H of the blood modified the reaction of the heart to caffeine. More extensive studies have since been made in this laboratory to ascertain whether variations in the P_H of the blood produced similar effects on the response of the heart to other substances. In the present report which deals with the results obtained in experiments with mercury succinate, observations have also been made to determine whether the reaction of the heart to the alteration of the P_H of the blood was the same after and before the administration of mercury. In addition investigations have been conducted on the effect of respiratory changes on the action of mercury.

The experiments were performed on cats anesthetized with methane. Mercury succinate was given intravenously each cubic centimeter containing the equivalent of 0.2 mg. mercury, the injections being made into the femoral vein from a burette at intervals of fifteen minutes. To reduce the P_H one and more rarely 0.25 to 0.5 per cent hydrochloric acid or 2 to 5 per cent sodium acid phosphate or both were administered in the same way. For increasing the alkali of the blood 2.5 per cent sodium carbonate was injected. The P_H of the blood was ascertained by the same method as in the investigation referred to above.

In studies with mercury compounds made some years ago in this laboratory it was found that it produced a characteristic effect on the circulation. After 2 to 4 mg. of mercury, in the form of succinate were injected intravenously, the blood pressure fell suddenly almost to the base line in most experiments, and after one to three minutes an equally sudden rise of the blood pressure occurred. This peculiar effect was usually observed after a latent period of about three minutes. The effect of each additional injection, after the attack once occurred, was increased both in severity and duration and sometimes recurred at frequent intervals. When the mercury salt was given to cats with the heart exposed by removing the anterior wall of the thorax and under artificial respiration larger doses were tolerated but, as before, the attacks occurred regularly after about the same interval and lasted two to three and sometimes four minutes or longer.

The behavior of the heart was as follows. The attack was ushered in by depression of the auricles which very frequently amounted to complete inhibition, the ventricles being also depressed, but not nearly to the same extent as the auricles for they usually contracted though the beats were

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very much slower and not much decreased in strength. The depression of the ventricles, however, was also considerable in some experiments. Besides cardiac depression we also noticed heart block, dissociation, group contractions and extrasystoles. These, it may be recalled, were described by Salant and Kleitman² in experiments on the isolated frog and turtle hearts when perfused with mercury salts. It may be pointed out, however, that the heart of these animals differed in some respects in its reaction to mercury from that of the cat heart *in situ*. The auricles in the frog and the turtle continued to beat some time after the ventricles stopped. Exactly the reverse occurred in the cat. Of interest also is the observation that delirium cordis noticed in the turtle heart, seldom occurred in the cat.

The time of appearance of symptoms of poisoning by mercury in cats while taking place regularly after an interval of about three minutes grew less after several doses have been given until the latent period was reduced to a few seconds. The duration of the individual attacks was, on the contrary, increased. Thus in one experiment the first attack lasted three minutes but the duration of the sixth was eight minutes. In some experiments repeated attacks occurred when the minimum toxic dose had been exceeded.

The total amount of mercury required to provoke the typical symptoms varied considerably, but was never less than 3 mg per kg and only in one experiment was the effect obtained with this amount. In the rest of the experiments this occurred after 4 to 5 mg per kg and sometimes 7 to 8 mg per kg have been injected before the first definite sign of mercury poisoning was observed. The fatal dose showed much less variation. Arrest of the heart was usually produced by doses of 7 to 9 mg per kg, but in one experiment the fatal dose was 11 mg and in another more than 14 mg per kg (Table I). The probable reason for the increased resistance in these experiments will be discussed later.

Quite different was the effect of mercury when the P_H of the blood was artificially changed by the intravenous administration of sufficient amounts of acid or alkali. Although the action of mercury was not always qualitatively different from that produced in controls the quantitative difference was often very considerable. This applies particularly to experiments with acid and mercury. We observed early in this investigation, while it was still in its preliminary stage, that the administration of 1 mg of mercury per kg given after a moderate amount of acid promptly arrested heart action. As the first test was made on a cat that had already received several doses of mercury, an experiment was carried out in which a sufficient amount of acid was given before the administration of mercury was begun. Ten cc of 1 per cent hydrochloric acid were injected into a cat weighing 33 kg. Heart action was depressed, the auricles being inhibited for about fifty seconds but the heart completely recovered within three minutes after the injection of acid. The introduction of 1 mg mercury per kg as succinate, into the femoral vein paralyzed the heart immediately after the injection was finished. Although the same amount of mercury in proportion to body weight proved to be less effective in other experiments, indisputable evidence of decreased resistance produced by acid was obtained. One milligram mercury as suc-

TABLE I
SHOWING TOXICITY OF MERCURY
TOTAL AMOUNT OF MERCURY PER KG IN MILLIGRAMS

	EXP	SIMPLE DEPRESSION	MARKED DEPRESSION AND IRREGULARITY	DEATH
MERCURY AFTER ACID	603	1		
	609	1		
	613	1		
	91			1
	592		2	
	595		2	
	601		2	
	614		2	5
	611		2	4
	612			3
HYDROCHLORIC ACID AND ALI ALI BEFORE MERCURY	604		2	
	606		1	
	590		7	
	594		3	
	597		8	9
	596		4	7
	597		4	
	601		6	14*
	602		5	11†

Survived 8 mg per kilo
† Artificial respiration increased

minute, per kg when injected after hydrochloric acid greatly depressed the auricles, and the ventricles without producing however cardiac irregularity. That the increase of the P_{H_2} of the blood rendered mercury more toxic was shown in nine other cases. The typical effect which, as described above, consisted of cardiac depression irregularity and very often arrest of the heart, was produced in seven experiments with 1 to 2 mg of mercury per kg. In another cat this was observed only after the third injection of mercury or after a total of 3 mg per kg. On the other hand 3 mg and indeed even 1 mg was fatal in two cats of this series. That the toxicity of mercury may also be greatly increased by weak acid was shown in one of these experiments. In this case 0.5 instead of 1 per cent hydrochloric acid as in the other experiments was given before mercury. The P_{H_2} of the blood being reduced 0.13. The first dose of mercury succinate provoked an attack of the heart, the auricles being inhibited for nearly three minutes while the contractions of the ventricles during this time were greatly decreased in force and frequency.

We observed, however, that in some experiments larger amounts of mercury were required to produce the effects described in spite of the administrations of acid. It occurred to us that the amount of air introduced into the lungs might be a causal factor. New experiments were therefore instituted to test this suggestion. The results obtained indicated that the toxicity

of mercury was affected by varying the pulmonary ventilation. When this was greatly increased mercury was less toxic notwithstanding the administration of acid, this being especially the case when dilute acid was injected. On the other hand when pulmonary ventilation was lessened, mercury after acid was much more toxic. The effect of pulmonary ventilation was also studied in experiments in which mercury alone was given. It was found that when respiration was increased much larger amounts of mercury could be given. In one experiment more than 14 mg per kg were tolerated, in another 11 mg per kg, whereas the maximum fatal dose under moderate artificial respiration varied between 7 and 9 mg per kg (Table I). The beneficial or detoxicating effects of increased pulmonary ventilation was also observed even after alkali had been administered. The explanation of the effect of the greater volume of air on mercury poisoning when the alkalinity of the blood is increased will be discussed later.

Brief descriptions of the following experiments will help toward a better appreciation of the results discussed above.

Experiment 613—Cat, male, weight 3 kg. Urethane anesthesia, P_H of the blood was 7.47, 2.53 P.M. to 3.26 P.M., seven injections 5 cc each were made of 1 per cent hydrochloric acid. The last injection depressed the auricles and slightly also the ventricles, but no significant changes occurred after the previous injections. Fifteen cc 1:5000 mercury succinate given two minutes after the last dose of acid, when the heart began to improve, produced considerable depression of the auricles and to a lesser extent also of the ventricles. The heart recovered completely in 4 minutes. Three more injections administered 15 minutes apart, made subsequently, had no effect on the heart but the fifth injection after acid produced cardiac depression again, from which the heart recovered.

In another experiment mercury was very toxic in spite of increased respiration. This experiment differed, however, in several respects from the others of the series. Heart action was not appreciably affected by acid of which 0.15 cc per kg was given, but a few minutes after 10 cc blood were drawn from the carotid artery, heart action became very weak, the contractions of the auricles being very feeble. After some improvement, a dose of 1 mg of mercury was given. This depressed the auricles, but did not affect the ventricles. The next injection was more effective, while the third was fatal. Although the results of this experiment show that increased P_H (the P_H after the last injection of acid was 7.15) increased the toxicity of mercury in spite of increased pulmonary ventilation the typical changes produced by mercury were absent.

As the results of this experiment might have been accidental two other cats were given mercury administered after acid while maintaining only a moderate degree of pulmonary ventilation. After sufficient amounts of acid had been injected mercury produced effects similar to those observed in our earlier experiments. In one cat the second dose of mercury succinate caused moderate depression, the third was followed by a mild attack, typical of mercury, but the heart recovered. The fourth produced an attack which lasted ten minutes. This, too, was followed by recovery. The next injection was rapidly fatal. The toxicity of mercury was even greater in another case, an attack, though mild, occurred after the second injection. This was more severe after the third and was fatal when the fourth dose was given.

That increased P_H of the blood may become very harmful was shown when acid was injected after mercury. We observed in one of our earlier experiments that small amounts of acid given after the administration of mercury greatly depressed the heart, especially the auricles, which in several instances stopped contracting. In one experiment this occurred when 2 cc 1 per cent hydrochloric acid were injected after 4 mg mercury had been

given. In the same experiment two doses of 5 cc of the acid injected two minutes apart before mercury, was without effect on the auricles and caused slight temporary depression of the ventricles. These injections of hydrochloric acid, it may be remarked, were given after 49 cc 2.5 per cent sodium acid phosphate. Similar effects were noticed in other experiments when mercury was followed by acid. In one cat which received two doses of mercury of 1 mg per 1 g, 5 cc 1 per cent hydrochloric acid depressed the auricle and to a less extent also the ventricle. The results were corroborated when the same dose was repeated four minutes later. In each case the ventricles recovered but the effect on the auricles persisted. After a lapse of another four minutes the same amount of acid was given. This rapidly depressed both the auricles and ventricles. The auricles were paralyzed within one minute after the injection while the ventricles continued to beat feebly one minute longer. The effect of acid was even more drastic in another experiment. Six injections of 5 cc each of 1 per cent hydrochloric acid were given three to four minutes apart. In no case did any appreciable change in heart action occur which was probably due to the high alkalinity of the blood in this cat. Nine cc of mercury succinate or 5 cc per 1 g were then injected. This depressed the auricles slightly also the ventricles immediately after the injection. Irregularity which was transitory, developed later. Both recovered, however in about three minutes after the injection. Ten minutes after the mercury another injection of 1 per cent hydrochloric acid was begun. This stopped the heart promptly when scarcely 5 cc were given. In several other experiments which were performed later the same results were obtained and in one of these 2 cc 1 per cent acid given three minutes after a dose of 1 mg mercury per kg paralyzed the heart while two injections of the same amount of acid when given before the last dose of mercury produced only a slight transitory depression.

In this connection the results of two experiments may be given in which acid was injected simultaneously with the usual dose of mercury. In both cases heart action was promptly depressed whereas the same amount of acid alone administered before mercury produced very moderate depression in one and practically no effect in the other experiment. Since some injections of mercury given subsequently had no effect it is evident that the changes observed were due to the combined action of acid and mercury.

Tests were also made to ascertain the influence of increased alkalinity of the blood on the reaction of the heart to mercury. Although the effect was not as striking as with acid the evidence obtained indicated increased resistance. The protective action of alkali was manifested in some experiments when the typical attacks following the administration of mercury were suppressed by the subsequent injection of sodium carbonate. In one experiment symptoms of mercury poisoning developed after a total of 2 mg mercury per kg had been given. Several injections of carbonate were then made and the P_{H} of the blood which was now determined showed that it was distinctly greater than at the beginning of the experiment. Three more injections, of 1 mg mercury per kg failed to provoke an attack. After the administration of sufficient amount of acid to reduce the alkalinity to normal, another dose

of mercury, the same size as before, was given but this failed to produce the usual effect. In another experiment similar results were obtained with alkali and mercury. The results, however, were not as uniform as in the case of acid.

We observed that alkali may also decrease the resistance to mercury. In this case 50 cc 2.5 per cent sodium carbonate were given at the beginning of the experiment before the administration of mercury. The first injection was without effect, but the second dose of mercury produced the characteristic changes in the heart. Determination of the P_H showed that the blood was markedly alkaline and well above normal. In another experiment in which carbonate was given after a total of 7 mg mercury per kg had been injected, cardiac depression occurred, the effect being most marked on the auncles.

DISCUSSION

That the effect of mercury on the heart was greatly influenced by altering the hydrogen-ion concentration of the blood was abundantly shown in these experiments. It should be added, however, that a considerable variation in the size of the effective dose of mercury was observed in some cases although there was little or no difference in the P_H of the blood. The interesting observation, however, was made that the toxicity of mercury was greatest when the P_H was 7.35 to 7.15 and also when it reached a value of 7.65. Furthermore our studies indicate that it is not so much the absolute amount of hydrogen ions in the blood which is the determining factor as the change in each individual case. The same increase or reduction in the P_H of the blood may be equally effective in modifying the action of mercury.

As pointed out in the preceding pages, increased pulmonary ventilation played an important rôle in modifying the effect of mercury. This is readily explained in view of the lessened amount of hydrogen ions retained by the blood. Evidence was obtained, however, showing that the increased tolerance was not wholly due to greater alkalinity of the blood for heart action was greatly improved by increased pulmonary ventilation after the injection of sodium carbonate. Also, in experiments in which mercury alone was given, increased respiration was associated with a pronounced augmentation of tolerance. Whereas the fatal dose of mercury was ordinarily 7 to 9 mg per kg this was increased to 11 to 14 mg per kg upon increasing pulmonary ventilation. Hence the greater amounts of oxygen which was thus provided probably constitute an additional factor in raising the threshold of tolerance for mercury.

The increased toxicity of acid after mercury, described above, may be explained by the presence of mercury in the blood and also in the heart at the time of the injection of acid since this metal is very slowly eliminated and tends to be cumulative. It is probable, therefore, that the heart is injured and is thus more sensitive to acid. This would also explain the behavior of the heart when the two compounds are given simultaneously. We noticed in some cases, particularly when dilute acid was given, mercury injected subsequently sometimes behaved as in experiments without acid. This we believe may be accounted for by the rapid neutralization of acid by the alkali in the tissues and elimination by the lungs and kidneys.

SUMMARY

1 Small amounts of mercury in the form of succinate, administered intravenously into anesthetized cats produce cardiac irregularity, depression and arrest of heart action, which occur after a latent period of about three minutes and last several minutes. The latent period decreases and the duration of the attack increases with successive injections of mercury. Complete recovery may occur even after prolonged cessation of contraction.

2 Acid given in sufficient quantity before mercury greatly increases the toxicity of mercury. The amount of mercury necessary to produce the characteristic changes in heart action may be only half to a third of the dose required to cause the same effect without acid.

3 Very small quantities of acid are toxic even after moderate amounts of mercury.

4 Alkali confers protection against mercury, provided moderate amounts only are given. If sufficient alkali is introduced to increase the P_H of the blood by about 0.2 above normal mercury becomes more toxic.

5 Increased pulmonary ventilation increased the resistance to mercury. It was suggested that the effect was due to a decrease of the P_H of the blood as well as greater amounts of oxygen.

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³Salant. *Jour Am Med Assn*, 1922, lxxix, 2071.

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THE HEMOLYTIC PROPERTIES OF THE AUTOLYSIS PRODUCTS OF GASTROINTESTINAL MUCOSA, CONSIDERED IN CONNECTION WITH THE PROBLEM OF PERNICIOUS ANEMIA*

By BEAUMONT S. CORNELL,† M.D., BALTIMORE, MD

NYFELDT¹ showed that the autolysis products of various intestinal bacteria, particularly *B. coli*, had a definite hemolytic effect when injected into rabbits, and produced in 9 rabbits out of 15 a marked macrocytic anemia with megaloblastic bone marrow changes. To attempt to relate such experimental findings to the etiology of pernicious anemia reflects, perhaps, the viewpoint expressed by Adams,² when he suggested that this disease was due to subinfection by special strains of the colon bacillus group.

It has long been recognized that the autolysis products of various bodily tissues are not only toxic but to some degree hemolytic. Mason and Davidson³ showed that liver and spleen autolyzing in the peritoneal cavity of dogs exerted a profoundly toxic influence resulting frequently in early death and, in any case, affecting quite definitely the chemistry of the blood. Bradley⁴ reviewed the whole question of tissue autolysis and suggested that autolytic processes may be concerned more intimately than now believed in the mechanism of disease.

In pernicious anemia, quite apart from any stated process, the gastrointestinal mucosa has long been suspected of giving rise to the disease. Howard⁵ stressed this aspect of the study in 1911. Previously Berger and Tsuchiya⁶ isolated from the intestinal mucosa in persons dead of the disease a lipid fraction which was capable, on parenteral injection into animals, of producing a macrocytic anemia, and was found to be ten times as potent as a similar fraction from other corpses. By producing an artificial diarrhea in dogs and then extracting their intestinal mucosa, a very similar lipid fraction was obtained. Cornell⁷ noted during an attempt to implant *B. welchii* in dogs' intestines by feeding massive cultures of the organism, that the initial diarrhea which resulted was soon followed by an anemia of transient character but showing definite macrocytic morphologic disturbance of the red blood cells. Burns and Dixon⁸ seek to relate the severe diarrhea occurring in 30 per cent of cases of pernicious anemia to some alteration in the lipid metabolism of a casual nature. Haden⁹ suggests that a pathologic state of the intestinal mucosa may be capable of giving rise to a toxin which may cause pernicious anemia. It is unnecessary to remind the reader of the severe pernicious-like anemia which may attend gastric or colonic cancer, or sprue, or diphtheriocephalus infection, in all of these diseases the intestinal mucosa is either the site of malignant hyperplasia or is in close proximity to an invading parasite.

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Seyderhelm¹⁰ produced a microcytic anemia in dogs by artificially instituting a chronic stenosis of the lower ileum. Meulengracht¹¹ has collected eight instances of pernicious anemia associated with intestinal stricture. Chapman and Duft¹ report the disease in a woman who previously had what appeared to be paralytic ileus. Logan¹ found the *balantidium coli* in the stools of a group of cases of pernicious anemia. All these various observations may conceivably be overemphasized and their significance overrated but it is obvious that one of their common denominators is the intestinal mucosa.

Such suspicion of the digestive tract mucosa as a possible etiologic factor in the disease which but vaguely formulated is strengthened by the constitutional absence of function on the part of the gastric mucosa in all cases. Again, the mucosa of the tongue undergoes atrophy in 45 per cent of cases. Atrophy is not an invariable finding in the gastric mucosa (Faber and Bloch,¹⁴ Passey¹) but absence of function is virtually a constant finding. There is no evidence that the intestinal mucosa undergoes atrophy or even infection. The gastric mucosa always presents inflammation whether atrophy is present or not.

The general problem arising from these considerations is first of all whether the gastrointestinal mucosa is the seat of any abnormally active process. The further problem of whether any such process is related etiologically to the disease is somewhat further shield to inquire.

There is no indication that autolysis of this membrane occurs in pernicious anemia to any significant extent. Autolysis is a self digestion of a cell due to the activity of its intracellular enzymes and occurs when the normal physiologic balance of the cell is sufficiently upset by interference with its blood or nerve supply.

The present investigation was undertaken in order to gain information regarding the effect on the blood of the autolysis products of gastrointestinal mucosa. It includes *in vitro* and animal experiments. Since the protocols cover 100 pages of typed notes and since the results are not sufficiently important to require expanded descriptions they will be presented in abbreviated form.

RESULTS

Saline extracts of fresh mucosa of the stomach and duodenum of the rabbit have no hemolytic or size altering effect on the washed erythrocytes of the same animal when incubated at 37.5° C for one hour. When portions of rabbit's stomach, liver, duodenum and spleen are allowed to autolyze at 37.5° C for 18 hours the digests in isotonic saline, especially those of the stomach, duodenum and spleen when mixed with washed rabbit erythrocytes and incubated for one hour at 37.5° C exert a peculiar effect on these erythrocytes *viz* they cease to retain the globular shape which they assume in saline alone, and expand to a normal disc shape but of wider diameter than normal. Commercial pepsin in saline exerts no such effect on rabbit erythrocytes. It was also determined that a change in the P_H of a normal saline solution over a range from 5.2 to 8.4 had no such form altering effect on these cells. The digests of duodenum, liver and stomach also exerted a definite hemolytic effect

on washed rabbit cells, while that of spleen did not. The two factors to be ruled out were the toluol used in the digests and the question of infected digests. Toluol itself exerted no form-altering or hemolytic effect on washed erythrocytes. The digests from which the toluol was completely evaporated were just as active in both respects as before. Aerobic and anaerobic cultures of the digests showed only a gram positive bacillus, the cultures of which failed to exert any influence on the erythrocytes. It appeared therefore that digests of rabbit duodenum, stomach and liver, made under toluol, for 18 hours, exerted a form-altering and hemolytic effect on washed erythrocytes *in vitro*.

The factors in these digests responsible for the hemolytic and form-altering effects were not active enzymes since boiling them did not remove the effect, nor were they precipitated with the protein. Their effect could be quite easily neutralized by the addition of fresh or inactivated human or animal blood serum.

The injection into rabbits, subcutaneously or intravenously, of the digests of rabbit's or cow's duodenum produced mild anemia, slow in developing, characterized by a fall in the hemoglobin percentage and the red blood count, leucopenia, blood platelet reduction, and a moderate degree of macrocytosis. Practically no difference resulted whether these digests were first boiled or given unheated, the blood picture resulting suggested merely a toxic depression of the hemopoietic tissues.

A somewhat elaborate division of the digest of cow's duodenum into 4 lipidal fractions was made, but none of these separately, nor in any combination, produced more than a macrocytic, aplastic anemia when injected parenterally into rabbits.

It was then sought to determine the effect on the blood by placing freshly removed rabbit organs within the peritoneal cavity of rabbits. Carefully determined equal weights of liver, spleen, stomach wall, duodenal wall, heart muscle, kidney, and skeletal muscle were used, each being placed in the peritoneal cavity of rabbits of equal weight, the peritoneum closed and the blood observed for some days subsequently. These experiments were thrice repeated and the following results noted.

1 The greatest abnormalities in the blood, when such abnormalities occurred, were noted in all cases on the fourth day after operation.

2 Slight oligocythemia was produced in all rabbits except those receiving liver and skeletal muscle. In both these cases the red blood counts were increased. Marked oligocythemia occurred in those rabbits receiving duodenum and stomach, sometimes to the extent of 30 to 50 per cent of the original blood count.

3 The hemoglobin percentage was reduced in all rabbits except those receiving kidney, in which case it was increased. Rabbits receiving duodenum and stomach showed most pigment reduction (from 10 to 30 per cent).

4 All rabbits except those receiving kidney and liver showed leucopenia. Those receiving kidney and liver showed some increase of leucocytes. The most marked leucopenia developed in those receiving duodenum (loss of 41 per cent), spleen (loss of 62 per cent), and heart muscle (loss of 66 per cent).

5 The only morphologic changes in the red blood cells occurred in those rabbits receiving duodenum and stomach. In these cases, the macrocytosis on the third to fifth days was often extremely marked, sometimes in the presence of anemia and sometimes with little accompanying anemia. Active regeneration of the marrow was indicated by increasing reticulocyte counts from the second to the fifth day in these cases.

It was determined by 50 control experiments that this anemia with macrocytosis produced by introducing stomach or duodenum into the peritoneal cavity did not depend on autolytic activity. The technique uniformly employed throughout was as follows:

The fresh stomach or duodenum (or other organ) was removed, opened, washed in running sterile saline, weighed and then placed in toluol till ready to use. It was placed in toluol to kill microorganisms and usually remained there for thirty minutes. Then it was removed from toluol, thoroughly washed again in running saline and finally placed in the peritoneal cavity of a rabbit.

In the control experiments it was discovered that the stomach or duodenum could be heated to 50 C. for 30 minutes after removal from toluol, without in any way interfering with the effect on the blood of the rabbit into which it was subsequently introduced. Furthermore, the mucosal scrapings, either heated or unheated produced the same effect on the blood. But if no preliminary contact with toluol was permitted to take place, the organ or its mucosal scrapings had no effect on the blood whether they were heated or unheated. On the other hand toluol by itself was repeatedly injected intraperitoneally in 0.5 cc. doses without effect on the blood.

The most reasonable explanation of the phenomenon was as follows: There exists in the mucosa of the stomach and the duodenum some thermolabile substance which is capable on absorption of depressing bone marrow activity and causing abnormal blood formation provided it is previously extracted with toluol at room temperature. Presumably toluol removed an inhibitory substance. Our attempts to recover this inhibitory substance from the toluol were unsuccessful.

CONCLUSION

The mucosa particularly of the stomach and duodenum after preliminary treatment with toluol is capable on intraperitoneal introduction, of causing a marked macrocytic anemia. There is no indication that this phenomenon has any relation to the problem of pernicious anemia.

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COMPLEMENT-FIXATION REACTIONS, USING ANTIGENS PREPARED FROM THE AUTOLYSIS PRODUCTS OF STOMACH, DUODENUM, AND OTHER ORGANS, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA BLOOD*

By BEAUMONT S CORNELL,† M D, BALTIMORE, MD

AS I¹ previously pointed out, the mucosa of the stomach and duodenum, after preliminary treatment with toluol, is capable, on intraperitoneal introduction, of producing a marked macrocytic anemia in rabbits. Bone-marrow depression is quite evident in this anemia. The smear picture is somewhat suggestive of an early pernicious anemia smear, the macrocytes being characteristically circular rather than oval. The phenomenon is not considered to have any relation to the etiologic problem of pernicious anemia. An opportunity presented, however, of doing complement-fixation reactions on a series of pernicious anemia blood samples, using as antigens alcoholic extracts of the autolysis products of various organs, the results of which are given below, with controls.

The antigens used were made as follows:

Cow's, or human organs, were placed whole under toluol at 37.5° C for periods varying from 2 to 4 weeks. At the end of that period, the resulting material was evaporated to a paste in front of a fan, the paste collected and kept in the ice box.

The antigens were made usually by extracting the dried autolysis products for 2 weeks with absolute alcohol. Sometimes preliminary extraction with ether was done before the alcoholic extraction. In all, 35 different antigens were employed. Most of these antigens were moderately hemolytic but few were anticomplementary. Double titrations were routinely done to determine the dilution used, which was ordinarily 1 in 40, sometimes 1 in 60.

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Tests were carried out within 24 hours of taking the blood sample. The technique employed was a modification of the original Wassermann, each specimen being controlled by a tube omitting antigen. Double tests were done in all cases: (1) with inactivated serum, (2) with unheated serum.

Results.—1 Out of 75 cases of pernicious anemia in all stages of the disease, 2 only gave 4 plus positives and 14 gave from 1 plus to 3 plus positives with an antigen made from cow's duodenum autolysis products.

2 The only other positive reactions were obtained in (a) 2 cases of pulmonary tuberculosis with cavitation (complete) (b) 24 cases of syphilis in whom a positive Wassermann was present (10 complete and 14 partial fixation).

3 The balance of the cases were negative and included 7 cases of pulmonary tuberculosis with cavitation, 96 cases of syphilis with positive Wassermann tests, and 328 cases of various other diseases the bloods being obtained from the Wassermann laboratory of the Johns Hopkins Hospital and from Ludowood Sanitarium.

From these results it will be seen that the reaction has no specific value whatever, and may occur in pernicious anemia, syphilis and tuberculosis.

An experimental study of the reaction was undertaken. Nine rabbits from stock were taken and as a preliminary their sera were tested against 5 standard antigens made by alcoholic extraction of various cow and human organs. Each rabbit was then immunized over a period of a month by repeated injections of autolytic products, each rabbit always receiving the product of the same organ and different rabbits receiving always different organs. The organs used for immunization included all five organs used in making the five antigens. The results were as follows:

1 All nine rabbits were at the beginning negative to each of the 5 antigens.

2 All rabbits, after immunization, were negative to each of the same 5 antigens.

This is an indication that a positive reaction in a human being does not depend upon any type of immunologic response to products of autolysis.

The meaning of a positive reaction with such antigens is of course obscure and probably indicates some physicochemical state that may be shared by the three diseases mentioned—pernicious anemia, tuberculosis and syphilis.

CONCLUSION

The serum of patients with pernicious anemia, syphilis and tuberculosis occasionally give a positive complement fixation when an antigen prepared from the autolysis products of various organs is used. The significance of this reaction is quite obscure and probably depends on physicochemical factors of no practical importance.

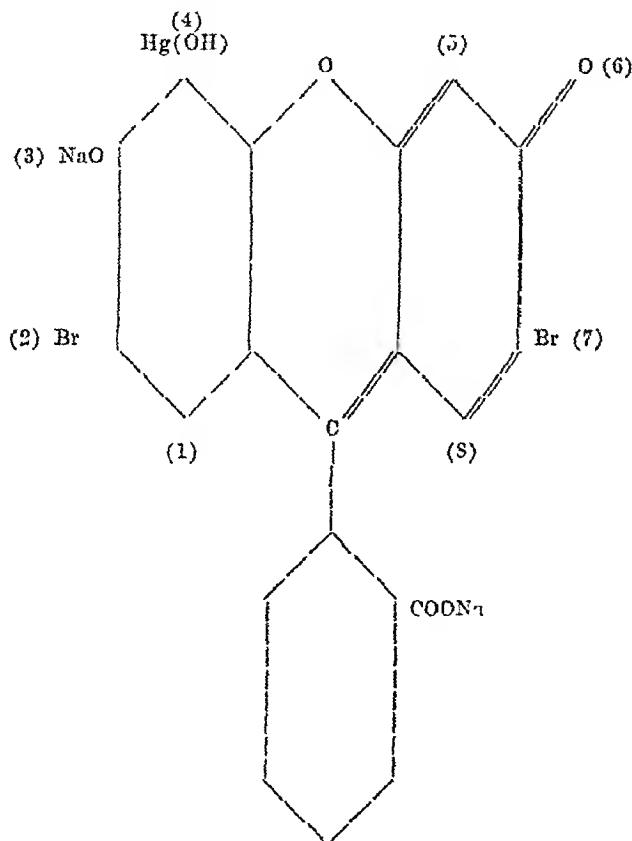
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NOTES ON SO-CALLED DIBROM-OXYMERCURY-FLUORESCIN SODIUM SALT (MERCUROCHROME-220 SOLUBLE)*

By BERNARD SALKIN, B SC, CHEMIST, BROOKLYN, N Y

WHEN dibrom-mercury-fluorescein was first described in the literature[†] the following structural formula was given, as representing that compound



The encircled figures represent positions as mentioned in the text

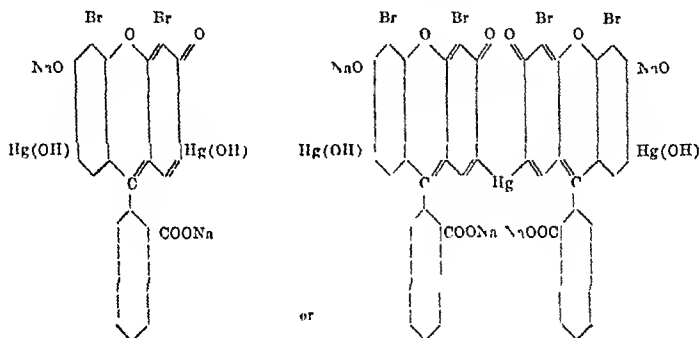
From the description of the method of manufacture given by White[†] there is no reason to believe that a pure product, or one having the above-mentioned structural formula, is obtained. That the reaction is allowed to continue until no mercury is present in the solution to react with ammonium sulphide, only indicates that all of the inorganic mercury (free Hg ions) has entered into complex or organic combination

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When bromine is added to fluorescein, in order to form dibromfluorescein, positions (4) and (5) are those *first* attacked on substitution, the hydrogen atoms at (2) and (7) being replaced subsequently³

Furthermore, when bromine is added to dibromfluorescein in order to form eosine, the reaction is of such a nature that the bromine substitutes in positions (2) and (7) simultaneously, no tribromfluorescein being formed. It has been shown by White and others that mercury acts like a halogen when substituting in fluorescein. Therefore, reasoning by analogy, it would appear that in the preparation of Dr. White's product, either a dioxymercury compound, or a sesqui compound is formed. In the latter case one of the mercury atoms is divalent and connects two molecules of dibromfluorescein.



As the reactivity of mercuric acetate is much less than that of bromine, it must be expected that the product of the reaction of the former with dibromfluorescein will consist of several components in equilibrium, as indicated above.

That such is the case was proved by experimental results. Several samples of the sodium salt of dibrom oxymercury fluorescein, stated by the manufacturer as having been prepared by Dr. White, were obtained. The mercury assay varied from 22.9 to 26.8 per cent on the anhydrous basis (powdered sample dried in a vacuum desiccator over sulphuric acid). (Theory for $C_{20}H_8O_6$, $HgBr_2$, Na is 28.7 per cent.)

Using a 2 gram sample (finely powdered) and 100 ml of 95 per cent ethyl alcohol, about 50 per cent of the material was dissolved. This material assayed only about 14 per cent of mercury on an anhydrous basis.

The residue from the first extraction was again treated with 100 ml of alcohol, and the extract yielded only 0.18 grams of material. Re-extracted as above the residue from the third extraction showed a mercury content of about 40 per cent. (Theory for $C_{20}H_8O_6$, $HgBr_2$, Na_2 is 41.4 per cent.)

Further treatment with alcohol of the residue after the third extraction yielded only about 0.18 grams per 100 ml of alcohol.

The same results were obtained using hot alcohol for extraction—the solution being allowed to cool to 20° C before being poured off from the residue—

as when the entire extraction was done at 20° C. Other experiments showed that even if only 50 ml of alcohol were used for 2 grams of material, about 1 gram was obtained in the first extract, the solubility of the residue being only 0.18 grams per 100 ml.

These experiments show that the product obtained by the method given by Dr. White is not a homogeneous compound. As it is known that dibromfluorescein is very soluble in alcohol, and an organic compound containing about 40 per cent of mercury would tend to be rather insoluble in the same solvent, it can readily be seen that, in all probability, in the process outlined by Dr. White, the dimercury-dibrom-fluorescein (or dioxymercury compound) is formed. When all of the inorganic mercury of the reaction mixture thus becomes fixed, a large portion of the dibromfluorescein remains unacted upon.

The writer had the original material and extracts examined in the spectrophotometer. The work was not done satisfactorily, as the samples were dissolved in alcohol instead of water. The results showed, however, that readings made on the residue, obtained after three extractions with alcohol, gave a curve very similar to that obtained for eosine. Readings made on the first extract gave a curve very similar to the one obtained for dibromfluorescein, while the curve obtained for the original product was midway between the two.

In none of the experiments did any metallic mercury separate, or were there any other signs of decomposition.

The method given by Dr. White for the analysis of the mercury compound of dibromfluorescein,² which is also found in *New and Nonofficial Remedies*, does not give results which can be checked even by the same analyst. Analyses varied as much as 1 per cent on duplicates, and 3 per cent on checks. That this was due to the technique of the method and not to the method itself was shown by the fact that with the writer's improved technique, checks and duplicates could easily be obtained, and the time required for an analysis was greatly reduced.

The following is the improved method and is applicable to the assay of organic mercurials and for mercury compounds containing organic material.

Into a 250 ml Erlenmeyer flask, weigh 0.3 to 0.35 grams of sample to be assayed. Dissolve in 10 ml of water and add 15 ml concentrated H_2SO_4 . Cover neck of flask with a small watch glass. Mix and add powdered $KMnO_4$ in small portions, until 2.5 grams have been added. Heat to incipient boiling. Cool, add 75 ml of water and then add oxalic acid to decolorize the solution and dissolve the precipitated manganese oxides. Only a slight excess of oxalic acid should be used.*

Filter the solution into a 600 ml beaker, add water to make a volume of about 300 ml, and pass in H_2S gas. When all of the mercury has been precipitated, warm the solution slightly to coagulate the mercuric sulphide, and filter on counterpoised filter papers. Whatman No. 40 filter paper has been found to be best for the purpose. Wash the precipitate by decantation.

*It has been found that any material excess of oxalic acid causes a precipitate of mercurous oxalate to form. Unless this precipitate is redissolved before precipitating the mercury with H_2S low results are obtained.

Dry the filter papers to constant weight in a hot air oven at 110° C
 Weight obtained multiplied by 0.8622 equals weight of mercury in sample

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STUDIES IN CALCIUM AND CARBOHYDRATE METABOLISM

I CALCIUM AND GLUCOSE TOLERANCE IN DIABETES MELLITUS*

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CONSIDERABLE work has been done on the subject of the influence of calcium on carbohydrate metabolism. The situation is, however, still in quite a nebulous state. Where a single mechanism is concerned in the production of a certain condition it is comparatively simple to study the effects of a drug on that condition and to explain its action; but where numerous mechanisms are concerned, as in disturbances of carbohydrate metabolism, it becomes increasingly difficult to determine the pharmacology of any drug that may be used. A review of the relationship between calcium and carbohydrate metabolism will be given.

Hyperadrenalinism (adrenalin injections) is associated with hyperglycemia, glycosuria, and a diminished tolerance for glucose; that is, the glucose tolerance curve is similar to that of a mild diabetic. In hypoadrenalinism (Addison's disease) the reverse is true. The effects of adrenalin appear to arise from stimulation of the terminal mechanism of the sympathetic fibers in the liver which control the glycogenic function. The calcium metabolism in hyper- and hypoadrenalinism has received scant attention. Mayer¹ could not find any increase in serum calcium in cases of rickets and spasmophilia as a result of adrenalin injections. Pulay and Richter² observed that the injection of massive doses of adrenalin in dogs leads to hypercalcemia. As to the influence of calcium on the disturbance in carbohydrate metabolism engendered by adrenalin, conflicting results have been obtained. Schrank³ observed that calcium will inhibit the glycosuria produced by adrenalin. Usener⁴ found that calcium prevented adrenalin hyperglycemia and glycosuria. This was also true two weeks after the splanchnic nerves had been sectioned. He concluded that calcium acts by depressing the sympathetic end plates which supply the liver, for calcium depresses the autonomic nervous system as well as all nerve tissues. Adrenalin by stimulating the sympathetic gives rise to increased glycogenolysis, glycemia and glycosuria; calcium by inhibiting the sympathetic would prevent such an action. Underhill⁵ has obtained quite

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reverse results. In his experiments calcium increased adrenalin hyperglycemia, it also augmented the glycosuria.

The thyroid gland exerts an influence on carbohydrate metabolism. Hyperthyroidism is associated with a diminished tolerance for glucose, hypothyroidism with an increased tolerance. Kummer⁶ found a negative calcium balance in one case of exophthalmic goiter. Castex and Sehtemgart⁷ found no changes in blood calcium in hyper- and hypothyroidism. Waldorp and Tielles⁸ found the blood calcium reduced in 26 subjects with thyroid disease and increased basal metabolism. The influence of calcium on the glucose tolerance in hyperthyroidism has not received any attention.

Von Noorden⁹ observed that acromegaly was frequently associated with diabetes mellitus. Conflicting results have been obtained as far as the calcium metabolism is concerned, some observers have found a retention, others an increased elimination. No attention was paid to the stage of acromegaly, hyper- or hypoactive. Their results cannot therefore be accepted seriously. The only work on the action of calcium is by Kuhl,¹⁰ who observed that it depressed the hyperglycemia produced by pituitin injections.

Parathyroidectomy produces a hypocalcemia. The majority of investigators have found a heightened irritability of the autonomic nervous system. Eppinger, Falta, and Rudinger,¹¹ also Marine,¹¹ observed a diminished tolerance for glucose in parathyroidectomized dogs but not in the tetany of man. Underhill and Blatherwick¹² observed that thyroparathyroidectomy causes hypoglycemia and that the normal glucose level is restored by calcium injections. They concluded that calcium may play an important rôle in maintaining the equilibrium of the blood sugar regulating mechanism during life. This problem is further complicated by the fact (Watanabe¹³) that guanidine, which is frequently increased in tetany, may cause a hypoglycemia.

Many investigators have found a physiologic decalcification in pregnancy. Hetenyi and Liebman¹⁴ observed that towards the termination of pregnancy there is a hypocalcemia and that during pregnancy calcium introduced into the circulation is rapidly withdrawn. Glucose tolerance tests (Labbe and Cherk¹⁴) gave mild diabetic curves.

Gerhardt and Schlesinger¹⁵ found an increased excretion of calcium in acidosis. Underhill⁵ observed that the injection of hydrochloric acid markedly augmented epinephrin hyperglycemia and glycosuria. Alkalosis, on the other hand, produced the reverse effect. Epstein and Felsen¹⁶ observed an increase in hyperglycemia in diabetics where acidosis existed, this was relieved by alkalis. This is also the finding of Underhill.¹⁷

The abstraction of calcium from the circulation gives rise to a heightened irritability of the autonomic nervous system (Chan and Froehel¹⁸). One would therefore expect a diminished tolerance for glucose. Kahn and Kahn¹⁹ observed that the introduction of tartrates intravenously produces a glycosuria. Underhill,⁵ on the contrary, found that the introduction of sodium phosphate into the circulation may cause a distinct diminution in blood sugar and also render less marked the hyperglycemia of adrenalin.

Usener⁴ observed that calcium depressed diuretin glycosuria but had no effect on phlorizin diabetes. Jacoby and Rosenfeld²⁰ found that the admin-

istration of calcium lactate had an immediate effect on phlorizin diabetes, the excretion of sugar falling to zero. Salant and Kahn¹ noticed that the administration of calcium to rabbits suffering from essential diabetes caused a cessation of the glycosuria and if the animal was previously fortified with calcium glycosuria never appeared.

Certain diseases such as pneumonia and tuberculosis are characterized by decalcification, this is especially marked in the latter. They are constantly associated with a diminished glucose tolerance.

The calcium metabolism in diabetes mellitus has received considerable attention. Von Mierzejewski² found an inordinate excretion of calcium in one case of diabetes mellitus. A year later he reported cases where the excretion of sugar was reduced by the daily ingestion of 10 grams of calcium phosphate.³ Von Noorden⁴ observed uniformly a negative calcium balance in diabetes. This was out of proportion to any increased calcium elimination that might be caused by an existing acidosis. Falta and Whitney⁵ found that pancreatectomy was associated with an immediate and rapid elimination of calcium. This was far in excess of that which could be accounted for by the mild acidosis present. The most elaborate work on the relationship between calcium and diabetes mellitus was performed by Kahn and Kahn.¹⁰ They first showed that out of five cases examined all showed a definite negative calcium balance. The patients were kept on a standard diet for a period of three days, during which time the glycosuria and glycemia were determined. On the same diet they were then injected intravenously with varying amounts of eighth molecular calcium chloride every few days. The glycosuria and glycemia were determined to observe the effects of the treatment. As a result of these experiments they concluded that calcium administration definitely reduces glycemia and glycosuria in diabetes mellitus. More recently the following work has been done. Davies, Dielens and Dodds⁶ observed that in rabbits the injection of insulin produced a decided increase in blood calcium, and Harrop and Benedict⁷ did not find this to be true in man.

A direct and accurate method of estimating the tolerance of an individual for glucose is the glucose tolerance test. A normal individual when given 175 grams of glucose per kilogram of body weight on a fasting stomach shows a blood sugar curve as follows: the maximum increase usually occurs at the end of one half hour and is never more than from 30 to 50 per cent above the fasting level, at the end of two hours the fasting level should be regained. No glycosuria should occur. In diabetes the curve is equally characteristic: the maximum increase is much greater and the fasting level is not regained for many hours. Glycosuria occurs depending on the height of the curve and the renal threshold of that individual for glucose. The indices of the severity of any case therefore are: the height and length of the glucose tolerance curve and the amount of glycosuria. There are other criteria that may be employed, namely, the changes in the difference in glucose concentration between arterial and venous blood and the changes in the respiratory quotient during a glucose tolerance test. The latter are not used in this work.

The influence of calcium on the glucose tolerance curve in diabetes was studied. Six cases suffering from diabetes of varying degrees of severity

were chosen. The procedure was as follows: each patient after fasting fourteen hours received 100 grams of glucose. Four blood specimens were taken: one, a fasting level immediately before ingestion, and three at the following periods, forty-five minutes, two hours and three hours after ingestion. The estimation of the blood sugar content was by the Kiamer-Gittelman method²⁸ in which blood is obtained by pricking the finger tip. It has been shown by Foster²⁹ that the sugar content of such blood is identical with that derived from the radial artery, in other words blood from the finger tip may be considered arterial blood. During the three hour period the urine was collected and its glucose content determined. Two or three days later (in Case 2, two weeks later) a similar procedure was carried out with the following modifications. Two blood specimens on a fasting stomach were taken one-half hour apart. Immediately after the first an intravenous injection of 1 gram (10 c.c. of a 10 per cent solution) of calcium chloride was given. The diet during the period of experimentation was unchanged.

CHART I

CASE NO.		1		2		3		4		5		6	
FL	8 A M	260		253		223		213				210	
	8 05	C ₁ *		C ₁		C ₁		C ₁		C ₁		C ₁	
F L	8 35	305	212	222	338	298	220	222	200	173	198	200	210
	8 40	GLUCOSE (100 GRAMS)											
45 Minutes													
post													
urine	9 25	444	427	386	546	355	355	355	426	360	293	309	296
2 Hours													
post													
urine	10 40	410	355	462	471	428	380	353	313	336	269	265	337
3 Hours													
post													
urine	11 40	370	305	320	404	395	392	333	313	297	240	217	254
8 40													
Urine†	to												
	11 40			236	230	162	118	75	80	45	24	26	18

*10 c.c. of 1 per cent calcium chloride intravenously

†Glucose content in grams

Figures for blood sugar are expressed in terms of milligrams per 100 c.c. of blood

An analysis of the results (see chart) reveals the following:

(a) The fasting blood-sugar level in Cases 1, 3, and 6 was not influenced by the calcium injection, in Case 2 there was an increase from 253 to 338, and in Case 4 a decrease from 213 to 200.

(b) Examination of the blood-sugar curves shows that in some cases they are not as high when calcium is given as in the control, in others the reverse is true. The conclusion is that calcium does not appreciably influence the curve.

(c) The urine was collected for three hours after ingestion and its glucose content determined. Three out of five cases showed a diminished output of glucose when calcium was given.

DISCUSSION

The literature on the relation of calcium to carbohydrate metabolism has been reviewed. It can be seen that there are many conditions in which there are

simultaneously disturbances in calcium and carbohydrate metabolism. The work that has been done is, however, fragmentary and inconclusive. No definite statements can as yet be made as to the pharmacology of calcium in carbohydrate metabolism. Much work of a systematic nature is necessary to clarify this subject.

The above study has been confined to the influence of calcium on glucose tolerance in diabetes mellitus. Von Möræzewski and Kahn and Kahn concluded from their work that the administration of calcium ameliorates the diabetic condition. The glucose tolerance test may be considered an accurate index of the severity of a diabetic. An intravenous injection of calcium does not produce any changes in the curve. This definitely contradicts the conclusions of Von Möræzewski and Kahn and Kahn.

It was observed by Cammidge and Howard²⁰ that the action of insulin on the utilization of carbohydrate is very materially increased by parathyroid extract. Collip²¹ states that the function of the parathyroid hormone appears to be that of a regulator of calcium metabolism and its action is primarily as a calcium mobilizer. It is capable of producing a sustained hypercalcemia. To contend that it exerts its influence on carbohydrate metabolism indirectly through its action on calcium metabolism is to speculate. This work will be continued and the action of parathyroid extract on the glucose tolerance test in diabetes will be studied.

SUMMARY AND CONCLUSIONS

1 A review is given of the literature on the relation of calcium to carbohydrate metabolism.

2 It appears to be definitely established that diabetes mellitus is associated with a negative calcium balance.

3 Intravenous administration of calcium chloride does not appear to influence the tolerance of diabetes for glucose as determined by studying glucose tolerance curves. This is at variance with the work of previous investigators.

4 This work will be continued employing parathyroid extract.

I am indebted to Dr. Max Lederer, director of the laboratory, for helpful suggestions and to Dr. Edmund Shlevin for the use of the cases in his clinic.

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ON COLORIMETRY *

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RECENTLY it was my privilege to review the manuscript of a paper 'On Nephelometry' by Dr. Hans Klemm of the University of Berlin. This paper appeared in a recent number of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE. After reading Dr. Klemm's manuscript it occurred to me that the readers of this Journal would be interested in a corresponding paper 'On Colorimetry'.

The use of color as a means of determining the amount of a given substance present has long been employed. For example, the determination of ammonium, nitrite, and nitrate nitrogen in water or of carbon in steel. Also color may be referred to an absolute index of color value, for example, by use of the Lovibond Tintometer or it may be determined by absolute analysis in terms of wave length of dominant hue or its complement and the percentage of white, for example, monochromatic analysis by means of the Nutting Colorimeter.

Colorimetric methods have rapidly increased in number during the past twenty five years, so that the list now includes many metals (aluminum, chromium, cobalt, copper, gold, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, titanium, tungsten, vanadium, etc.), many non-metals (arsenic as arsenate, boron as borate, carbon, hydrogen ion, nitrogen as nitrite, nitrate, and ammonium, oxygen both free and as hydrogen peroxide, sulphur and sulphide and as sulphate, phosphorus as phosphate, etc.), and a large number of organic substances which include nearly all classes of organic compounds (alcohols, aldehydes, organic acids, esters, phenols, carbohydrates, alkaloids, hemoglobin, etc.).

In general, colorimetric analysis consists in adding a reagent to a solution of the test substance in such a way as to produce a color whose intensity is proportional to the concentration of the test substance in solution. As a matter of convenience and also to insure greater precision and speed, special forms of apparatus have been developed for use in colorimetry. The methods employed in matching colors may be grouped under four heads: (1) standard series method, (2) dilution method, (3) duplication method, (4) balancing method.

METHODS OF MATCHING COLORS

Standard Series Method—In this method the sample solution contained in a glass tube (or cell) is diluted to a definite volume, mixed, and its color compared with a series of standards similarly prepared.

In some cases it is possible and may be more convenient, to prepare a

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series of permanent standards² by means of solutions and mixtures of solutions of certain colored inorganic salts, such as for example, cobalt chloride, ferric chloride and copper sulphate. If such a series is employed, each solution must, of course, be standardized against a known amount of the original substance and under identical conditions as maintained in the analysis of a sample of the substance. Care must be taken that the tint or shade of color is the same in the permanent standard as in a solution of the substance to be determined.

Sometimes it is a great convenience to use a series of colored glasses as standards. For example, cobalt glass has been found to match the blue color of certain vat dyes reduced by alkaline sodium hyposulphite. The use of these permanent standards proved a great help in reaction velocity studies³ on these dyes where it was necessary to make rapid determinations every few minutes and also because the reduced dyes are readily oxidized by air, and therefore a freshly prepared standard would be required for each determination. The plates of glass were standardized against known weights of dye reduced under standard conditions and were always placed in the colorimeter in a definite position to guard against introducing an error due to any irregularity in the glass. In using colored glasses as permanent standards great care must be taken that the tint of color exactly matches that of the test solution.

With a series of standards the amount of substances in the sample is obtained directly, since it is equivalent to the amount contained (or represented) in the standard which it matches in color intensity.

Dilution Method If the sample and standard solutions when placed in glass tubes, or cells, of the same diameter and observed horizontally through the tubes have the same intensity of color, obviously their concentrations are identical. Usually the solutions do not match in color intensity, and the darker one is then diluted until a match is obtained when the two are viewed horizontally through the tubes, i.e., through the same thickness of liquid. This process of comparison is called the dilution method. When sample and standard solutions match their concentrations are the same, and, hence, the weights of the substances in the two solutions are directly proportional to the respective volumes.

Duplicate Method This method is carried out as follows. The sample is placed in a glass tube, or cell, diluted to a definite volume and mixed. Water is put in a similar vessel and the same reagent, or reagents added as used to produce the color with the sample. The volume in this blank should be a little smaller than that of the sample solution. Next a relatively concentrated standard solution of the substance being determined is run into the blank from a burette until its color matches that of the sample solution, the final observation being made after the duplicate has been brought up to the same volume as the sample by the addition of distilled water and thoroughly mixed. The amount of standard solution required to make the duplicate is a measure of the amount of substance in the sample solution.

Balancing Method This method consists in placing the sample solution,

or an aliquot portion, in a flat bottom graduated tube and then running into another similar tube a standard color solution until the color intensities of the two are the same when viewed vertically through the length of the columns of liquids. When thus balanced the concentrations of the two solutions are inversely proportional to their heights (not volumes) in the tubes. Schreiner¹ points out that "curiously enough, the graduation into cubic centimeters has been carried over to the cylinders used in many of these instruments (balancing or plunger type colorimeters) when it is perfectly obvious that it is the height of the standard liquid which determines the strength of the unknown solution." Of course if both the sample and standard tubes have the same diameter and the bore is uniform throughout in each tube, then the two concentrations are also inversely proportional to their volumes when the colors are matched by the balancing method. Such uniformity in color tubes is hard to obtain and unnecessary. The use of the cubic centimeter scale in graduating the balancing or plunger type colorimetric apparatus is, moreover, wrong in principle and therefore should not be employed. A rational graduation into scale divisions (say centimeters) independent of capacity or uniformity of bore of the tube should be used.

Instead of placing the sample solution in the tube and changing the height of the standard column until the color intensity is the same as that in the unknown a measured height of standard solution may be placed in one tube and the sample solution run into the other until a color match is obtained.

The balancing method is by far the most speedy of the four procedures already mentioned. It is also the most accurate provided the proper conditions are observed.

The change in the height of a solution has been accomplished in a variety of ways e.g. (1) by dropping from a burette into one of the comparison tubes, (2) by providing one or both of the tubes with a stopcock near the bottom, (3) by connecting by means of a side tube at the bottom, with a reservoir which permits moving the solution up and down at will, and (4) by changing the height by means of an immersion prism or tube.

REQUIREMENTS OF THE COLORIMETRIC METHOD

In order to be able to employ a colorimetric method certain conditions must be fulfilled, chief among which are the following:

- 1 The color produced by the reagent must be characteristic of the test substance, or in case certain other substances produce the same color as does the test substance, these must be known to be absent.

- 2 The color produced by the reagent and test substance must be the only color present in the solution. In some cases the presence of a very small amount of a foreign colored substance in the sample solution may be compensated by using a standard with the same concentration of the foreign substance.

- 3 The sample solution must be colorless or if colored this color must be removed by the reagent or some other step in the procedure.

4 The sample solution must not contain any foreign substance which will give a color or precipitate with the reagent

5 The color produced by the reagent must be reasonably permanent, i.e., it must not fade so rapidly that an accurate color comparison is impossible. Under certain well-defined conditions it is sometimes possible to employ a fairly unstable color and still obtain a satisfactory quantitative measurement.

6 Neither the intensity nor the tint of the color produced by the reagent and test substance must be affected by the presence of reasonably relatively high concentrations of electrolytes likely to be present. In certain cases it is necessary to adjust very carefully the hydrogen-ion or hydroxyl-ion concentration before an accurate color comparison can be made.

To these conditions may be added certain others which are desirable in a colorimetric method but which are not always required.

1 The intensity of the color should be directly proportional to the concentration of the test substance.

2 The color should be one easy to distinguish and to match, for example, blue, red, green, etc. In this connection it must be remembered that an operator may have a dull or imperfect susceptibility to one color and still be able to match other colors with great precision; it is, therefore, important that he test himself thoroughly for each color by matching a standard against itself in several degrees of intensity. If concordant results are not obtained with a certain color, it is useless for him to go further with this color.

3 The method should be rapid, accurate, and sensitive. Frequently one or maybe two of these qualities are sacrificed in order to attain the more desired third quality. In general colorimetric methods are rapid and accurate and often are delicate enough to determine quantitatively one part of the test substance in several hundred thousand parts of water. Some are so sensitive that one part of test substance may be detected in a hundred million parts of water.

ACCURACY OF COLORIMETRIC METHODS

No general statement can be made as to the accuracy of colorimetric methods. Some colorimetric determinations have been brought to such a high degree of perfection that they far surpass gravimetric or volumetric determinations in accuracy. On the other hand, many colorimetric methods are only rough approximations. These approximate methods, however, serve a purpose, for in such cases we frequently have no other means of determining the substances, or often a very rapid method is necessary and a colorimetric procedure, although its results are only approximate, may meet this requirement. It is between the above two extremes of accuracy that most of the colorimetric methods lie. Attention has often been called to the extreme degree of accuracy attainable in colorimetric methods when properly carried out. "There is little doubt that their accuracy is more frequently underestimated than overestimated. This is due to a number of causes, chief among which are the inability on the part of many persons to judge colors accurately, con-

tamination while making the tests, the use of impure reagents, and the employment of faulty apparatus. Practice will do a great deal to enable one to make good comparisons, but it can never make up for a dulled or imperfect susceptibility to color." Great attention should be given to this point in using colorimetric methods.

SPEED OF COLORIMETRIC METHODS

As in the case of accuracy, colorimetric methods vary widely from the standpoint of speed. Some are extremely rapid, requiring only a few minutes, while others are very slow and tedious, especially if the highest degree of accuracy is desired. Often accuracy is sacrificed for speed. In developing colorimetric methods of analysis there have been two main objects throughout, namely, speed and the ability to estimate small amounts, both of which are common to many of the methods but not necessarily so. A colorimetric method may have speed and yet not be capable of estimating very small amounts. On the other hand some of the more recently developed colorimetric methods are fully as laborious and perhaps even more tedious than the gravimetric methods. Their one virtue is that they can be employed in determining amounts so small that gravimetric methods fail, and hence they present a means of attacking problems which hitherto have been impossible of investigation. In such cases speed is only of secondary importance.

LIMITS OF APPLICATION OF COLORIMETRIC METHODS

In general, a colorimetric method cannot be used when more than 1 or 2 per cent of the substance being determined is present without resorting to aliquot parts and using a portion of the solution of the sample instead of the whole. In the latter case it is, of course, necessary to measure the aliquot part as accurately as the sample was measured, otherwise the final result will be in error.

As for the lower limit of application it may again be pointed out that many colorimetric reactions are sensitive enough to detect one part of test substance in several million parts of water, and some will detect one part in a hundred million parts of water. Hence, by using a large weight of the sample material, or a large volume in the case of solutions and then concentrating by evaporation extremely small amounts may be determined. Of course, the size of sample that can be handled reaches a practical limit, for example, a long time is required for concentrating large volumes of liquids and great care must be taken to prevent contamination from dust particles, vessels, etc. Furthermore, various salts may crystallize out during evaporation and occlude some of the substance being sought, or a certain constituent which has no effect in the dilute sample may interfere when its concentration is increased.

In spite of the many requirements imposed upon colorimetric methods, it may be said that, in general, they are applicable to concentrations of 1 or 2 per cent down to one part in a hundred million, but these limits may be extended under proper conditions as already pointed out.

ERRORS OF COLORIMETRIC METHODS

In colorimetric analysis certain factors introducing variations and errors are frequently overlooked. Chief among these are variable sensitiveness of vision to different depths of color, the inability of many persons to judge colors accurately, and the bicolored nature of the solution.

Variable Sensitiveness of Vision to Different Concentration Hoin⁶ and his coworkers have made an experimental study of variable sensitiveness in colorimetry. They used solutions of CrO_4^{--} , Cu^{++} , and $\text{Cu}(\text{NH}_3)_4^{++}$ ions and showed that with equal depths at certain definite concentrations the comparisons in the colorimetric determinations of these ions can be made with greater ease and accuracy than at other concentrations. It is held by them that this relation is a "perfectly general one throughout colorimetry" and they suggest that "the curve of sensitiveness must be known in each colorimetric method if the method is to be used to greatest advantage and the results are not to be affected by errors of unknown magnitudes."

Imperfect Susceptibility of Color Another cause of error in colorimetry is due to the inability of many persons to judge colors accurately. Practice will do much to enable a person to make good color comparisons, but it can never make up for a dulled or imperfect susceptibility to color. Great attention should be given to this point, and an operator should test himself thoroughly for each color by matching a standard against itself in several degrees of intensity. If he fails to obtain concordant results with a certain color, it is useless for him to go further with this color. In this connection it should be noted that a person who cannot use one method with accuracy is frequently able to use another with great precision.

Fatigue and Eye Strain Care must be taken on the part of the observer to avoid fatigue and eye strain. Failure to take this precaution may introduce a serious error in otherwise excellent work. Especially is this precaution necessary when a long series of determinations is being made. In such a case the use of a dark room will aid greatly. Each observer must, of course, determine his capacity in this respect.

Bicolored Nature of Solutions Dehn⁷ has made a study of the "fallacies in colorimetry" and gives a full discussion of them. He points out that "in most colorimetric determinations, the depth of solutions of the color standards is matched by various depths and concentrations of the solutions to be estimated. usually we have two columns of solutions of unequal length whose tints are matched to the limit of sensitiveness of the eye and whose solute molecules are assumed to be the same in number. That the solute molecules are not necessarily the same, not only in number but in composition, may be concluded from studies of (1) then different optical distributions, (2) different equilibria resulting from ionization, (3) different equilibria resulting from hydrolysis and, (4) different equilibria resulting from chromoisomerisation." For a detailed discussion of these sources of error in colorimetry the reader is referred to Dehn's work.⁷

Summary of Errors in Colorimetry The various possible errors to be dealt with in colorimetry may be summed up as follows

- 1 Errors due to the inability of the observer to judge colors accurately
- 2 Errors due to variable sensitiveness of vision to different concentrations
- 3 Errors due to fatigue and eye strain
- 4 Mechanical errors of the colorimeter
- 5 Optical errors of the colorimeter
- 6 Optical errors resulting from varied light
- 7 Errors resulting from scale readings
- 8 Errors of dilution
- 9 Errors due to temperature variation
- 10 Errors due to varied times of standing
- 11 Errors due to varied quantities of reagents
- 12 Errors due to the presence of interfering substances
- 13 Errors due to variations in the hydrogen ion concentration
- 14 Errors due to the action of light

It would seem from the above list of the sources of errors in colorimetry that accurate and precise measurements by this method would be hard, if not impossible to obtain. Fortunately, however, most of these errors can be avoided, or reduced to a satisfactory minimum by carefully worked out procedures and good technique together with the use of a good colorimeter or carefully matched color comparison tubes. A number of colorimeters possessing optical and mechanical accuracy are described in the literature.

In conclusion we wish to emphasize the importance of studying the various sources of errors as they affect each colorimetric method employed.

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QUANTITATIVE DETERMINATION OF THE KAHN REACTION*

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THE need of a simple method of estimating quantitatively the changes in the serum of syphilitics is very generally recognized. The Wassermann reaction is quantitative only within narrow limits. A ++++ result is recorded alike for sera containing a moderate amount of reacting substance and for others containing a very large amount. It would be of interest to distinguish between these. The slight degrees of hemolytic inhibition recorded as + are found both in active syphilitic and in nonsyphilitic conditions. It would be most helpful in diagnosis if the degree of reaction which may be considered indicative of syphilis could be more precisely defined.

Efforts to render the Wassermann reaction more sensitive have succeeded in increasing the positive results obtained in syphilis but have also brought out more nonspecific reactions. These experiences have led to the belief that the change in serum which the reaction detects is quantitative rather than qualitative.¹ The following discussion is based on this hypothesis. It is also widely accepted, for reasons we cannot here present, that the complement-fixation reaction of Wassermann and the various precipitation tests for syphilis are but different methods for detecting the same change in the serum of syphilitics. It has been the aim of many investigators to devise a technique which would permit more accurate measurement of the extent of this abnormality.

STANDARD QUANTITATIVE METHODS

Three quantitative methods have received wide trial: the Sigma reaction of Dreyer and Ward,² in which the precipitation of a lipid solution is observed in a series of graded dilutions of serum, the Verneux reaction,³ in which the opacity of one lipid-serum mixture is optically measured, and Kolmer's modification of the Wassermann reaction,⁴ in which a complement-fixation test is carried out with graded dilutions of serum, using a carefully standardized hemolytic system.

The quantitative method of Kolmer has been much used in this country, and many competent observers have reported favorably as to its accuracy and specificity. It is admitted, however, that it is a complicated and time-consuming procedure. Any reaction, moreover, which depends upon the use of a hemolytic system, employs two reagents (blood cells and complement) which cannot be satisfactorily preserved and which present real difficulties in standardization.

Precipitation methods have the advantage of employing as the only reagent a lipid solution which can be preserved for a long time without appar-

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ent change in its reactivity. Evidence is accumulating that such tests can be made as sensitive and as specific as the complement-fixation test. It would seem, therefore, that they are more promising for quantitative work.

The Vernes reaction is based on the assumption that the degree of opacity in a serum lipid mixture will correspond to the strength of the precipitating serum. There is, so far as we know, no proof of this assumption save the fact that the strength of the reaction obtained follows a descending curve in cases that are responding to treatment. We have had no personal experience with the method, but Dr. L. H. Cornwall has been kind enough to carry out the Vernes reaction on a number of sera which we have tested by the Wassermann, Kahn and Sigma methods. In a small number of cases of treated syphilis a definite reaction was obtained by the Kahn and Sigma methods where the Vernes was completely negative. The number of sera compared is too small to warrant definite conclusions but the results indicated to us that the method was not sufficiently sensitive for following treated cases and discouraged us from making a personal trial of the procedure.

The Sigma reaction has been extensively employed in England. Careful studies by Dreyer and Waid Jones and others have established its reliability. A considerable experience with the method has convinced us of its value but has also impressed on us its technical difficulties. It seemed that a simpler method might be found which would give results equally consistent with the clinical findings.

The technique described by Kahn⁶ for his qualitative tests seemed to offer the basis for a quantitative procedure that would possess the essential advantages of the Sigma reaction and avoid many of its difficulties. The method of measuring the reagent is simple, no long incubation is required, the method for antigen preparation gives an extract which is almost invariably satisfactory, the reading of the reaction with proper illumination is relatively easy. In short, this method is less time consuming and being simple, offers fewer chances for error than does either the Sigma or the Wassermann.

QUANTITATIVE METHOD FOR THE KAHN REACTION

Kahn has described a quantitative method for performing his reaction.⁷ It includes the preparation of a preliminary series of dilutions of the serum to be tested, a complication of technique which it would be desirable to avoid.

It seemed that this method could be simplified by considering the principles involved. To express our concept of a strongly or a weakly reacting serum it is almost necessary to think of them as containing a larger or smaller amount of some reacting substance. The simplest method for determining this amount is to test a serum in a series of diminishing quantities against a constant quantity of the other reagents and to determine thus the smallest amount of serum necessary for a standard effect. If only one other reagent is concerned the same result can be obtained by increasing the quantity of reagent as by diminishing the quantity of serum, the only important factor being the ratio between the two.

In testing sera from syphilitics by the Kahn method it was found that reactions could be obtained over a range where the ratio of antigen to serum

varied from 1:40 to 5:1. If 0.01 cc of antigen were employed throughout (as in Kahn's method), to obtain a 5:1 ratio would require the setting up of a tube containing 0.01 cc of antigen and 0.002 cc of serum—a quantity too small to measure without preliminary dilution. If, however, the amount of antigen were increased to 0.05 cc, the 5:1 ratio could be obtained by the use of 0.01 cc of serum, which can be directly measured. On the other hand to obtain a 1:40 ratio with 0.05 cc of antigen would demand the use of 2 cc of serum, an impracticable amount in routine work. This difficulty could be avoided by using 0.2 of serum and 0.005 of antigen.

Accordingly, we set up our reactions in tubes containing the following amounts:

TABLE I
TITRATION OF KAHN REACTION

TUBE	ANTIGEN 1-1	SERUM UNDILUTED	SALINE* 0.9 PER CENT	RATIO DILUTED ANTIGEN SERUM
1	0.005	0.2	0.0	1-40 0.2-8
2	0.01	0.2	0.0	1-20 0.4-8
3	0.025	0.2	0.0	1-8 1-8
4	0.05	0.2	0.0	1-4 2-8
5	0.05	0.1	0.1	1-2 4-8
6	0.05	0.05	0.15	1-1 8-8
7	0.05	0.02	0.2	2-1 20-8
8	0.05	0.01	0.2	5-1 40-8

*The addition of saline interferes as Kahn has shown with these precipitations but is necessary to insure proper mixing of the small amounts of reagents used in the upper tubes. It may make the absolute unit values obtained too low but does not alter the relative value of the results.

In this way a sufficient range of varying ratios of the mixtures could be obtained, using quantities measurable without preliminary dilution and a total amount of serum (1 cc) that was not excessive.

The other factors in technique may be defined briefly as follows:

Serum—The age of the serum seems immaterial provided that it is clear and free from contamination. After a number of trials with various periods of inactivation it seemed that for most sera the most sensitive reactions were obtained by inactivation for one-half hour in a water bath at 56° C.

Antigen—Antigen was prepared and titrated by the method described by Kahn and used, as a rule, in a dilution of 1-1, with 0.9 per cent salt solution. In referring to the amount of antigen used in the tests described we mean the amount of the mixture of salt solution and alcoholic extract prepared before making tests.

Shaking—Immediately after the serum is measured into each series of eight tubes, the tubes should be shaken by hand to insure prompt mixing. As soon as from six to ten tests are set up, they are placed in a shaking-machine and rocked for 20 minutes. We have found convenient a device shown in Fig. 1, which is adapted from the shaking-machine described by Warnick.⁸

The bed of the machine is a wooden platform 12 by 24 inches with two upright cleats, the height of a test-tube rack. One cleat is fixed and the other secured by a bolt passed through a slot which runs the length of the

bed. From one to eight of the ordinary copper racks used in serologic work may be clamped between these cleats. The wooden bed is suspended by four straps made from an ordinary clock spring. It may be hung below a table, saving laboratory space. It is rocked by a piston running to an eccentric pin on a reducing gear which is in turn driven by a belt from an electric motor. A speed of about 100 to and fro swings a minute has seemed satisfactory.

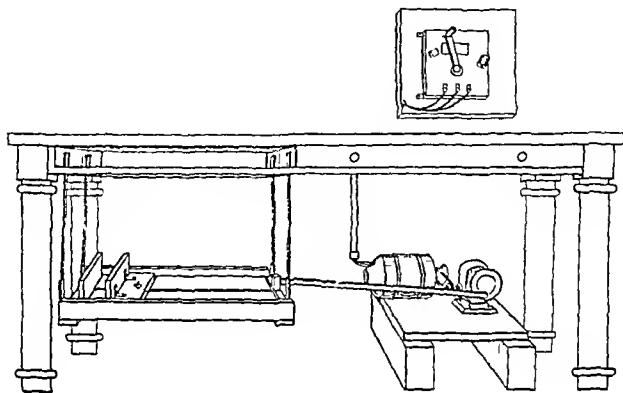


Fig 1—Shaking machine

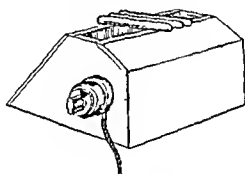


Fig —Illuminating device

READING THE REACTION

After rocking, 0.5 cc of salt solution is added to each tube, the rack is shaken for a moment to insure a mixture and allowed to stand 5 minutes before reading. If the tubes are allowed to stand for 24 hours, a more marked precipitation is sometimes observed. We have not used such reactions in quantitative work.

Reading has been greatly facilitated by the use of a small illuminating box shown in Fig 2. It consists of a wooden box lined with black velvet, the base measuring 8 by 8 inches, the top 8 by $2\frac{1}{2}$ inches, the height 5 inches. In the top is a slit $1\frac{1}{2}$ inches wide across which the tubes are laid horizontally so that the mixture runs out in a thin layer along the under side making a fine precipitate easily visible. A cylindrical electric bulb of the show case

varied from 1:40 to 5:1. If 0.01 c.c. of antigen were employed throughout (as in Kahn's method), to obtain a 5:1 ratio would require the setting up of a tube containing 0.01 c.c. of antigen and 0.002 c.c. of serum—a quantity too small to measure without preliminary dilution. If, however, the amount of antigen were increased to 0.05 c.c., the 5:1 ratio could be obtained by the use of 0.01 c.c. of serum, which can be directly measured. On the other hand to obtain a 1:40 ratio with 0.05 c.c. of antigen would demand the use of 2 c.c. of serum, an impracticable amount in routine work. This difficulty could be avoided by using 0.2 of serum and 0.005 of antigen.

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2	0.01	0.2	0.0	1-20 0.1-8
3	0.025	0.2	0.0	1-8 1-8
4	0.05	0.2	0.0	1-4 2-8
5	0.05	0.1	0.1	1-2 4-8
6	0.05	0.05	0.15	1-1 8-8
7	0.05	0.02	0.2	2-1 20-8
8	0.05	0.01	0.2	5-1 40-8

*The addition of saline interferes as Kahn has shown with these precipitations but is necessary to insure proper mixing of the small amounts of reagents used in the upper tubes. It may make the absolute unit values obtained too low but does not alter the relative value of the results.

In this way a sufficient range of varying ratios of the mixtures could be obtained, using quantities measurable without preliminary dilution and a total amount of serum (1 c.c.) that was not excessive.

The other factors in technique may be defined briefly as follows:

Serum—The age of the serum seems immaterial provided that it is clear and free from contamination. After a number of trials with various periods of inactivation it seemed that for most sera the most sensitive reactions were obtained by inactivation for one-half hour in a water-bath at 56° C.

Antigen—Antigen was prepared and titrated by the method described by Kahn and used, as a rule, in a dilution of 1-1, with 0.9 per cent salt solution. In referring to the amount of antigen used in the tests described we mean the amount of the mixture of salt solution and alcoholic extract prepared before making tests.

Shaking—Immediately after the serum is measured into each series of eight tubes, the tubes should be shaken by hand to insure prompt mixing. As soon as from six to ten tests are set up, they are placed in a shaking-machine and rocked for 20 minutes. We have found convenient a device shown in Fig. 1, which is adapted from the shaking machine described by Warnick.⁸

The bed of the machine is a wooden platform 12 by 24 inches with two upright cleats, the height of a test-tube rack. One cleat is fixed and the other secured by a bolt passed through a slot which runs the length of the

mixed with 0.125 c.c. of antigen under the conditions stated. If a serum contains one unit per cubic centimeter, 0.2 c.c. would then give a ++ reaction with 0.025 c.c. of antigen. If the same amount of serum gave the same reaction with twice the amount of antigen (0.05 c.c.), it must contain two units per cubic centimeter, and if one half the amount of serum (0.01 c.c.) precipitates twice the amount of antigen (0.05 c.c.), it must contain 4 units per cubic centimeter. The number of units in any serum could thus be determined by finding the ratio of antigen to serum in a mixture which shows a ++ reaction. In the cases just mentioned of sera containing from 1 to 4 units, the ratios of antigen to serum are 1 : 8, 2 : 8 and 4 : 8 respectively. We thus obtained the unit values shown in Table IV for ++ reactions.

We endeavored to calculate a series of interpolated values following Dreyer and Ward's method but were unable to find a constant relationship between the degree of reaction in one tube and that in the following tube. For example sera from one patient gave on successive tests the following readings:

TABLE III
RESULTS OF THREE SUCCESSIVE TESTS

TUBE	1	2	3	4	5	6	7	8
March 31	4	4	3	2	0	0	0	0
April 7	4	4	4	4	0	0	0	0
April 21	4	4	4	3	0	0	0	0

It will be seen from the readings of tubes three and four in the first test that reducing the proportion of serum by one half weakened the reaction from +++ to ++. On the other hand in tubes four and five of the third test, reducing the serum by one half weakened the reading from +++ to zero, and in the second test, from ++++ to zero. This is a typical example and illustrates the impossibility of finding in our readings a correlation between the strength of the serum and the coarseness of the precipitate. This may be due, in part, to the difficulty in defining precisely the varying degrees of reaction but at least it indicates that it would be safer to base our quantitative estimations on the amount of serum necessary to produce a reaction rather than on the intensity of the reaction obtained.

We determine, consequently, the unit values by reading the last tube which shows any precipitation and disregarding the preceding tubes. If the reading is ++, it is given a unit value as defined above. If it is +++ or ++++, it is arbitrarily increased by 25 or 50 per cent, and if + decreased by 25 per cent. This does not express the exact quantitative relationship but serves to indicate, for example, that a serum showing +++ or ++++ in the third tube and negative in the fourth is stronger than a serum giving a ++ in the third tube and weaker than one giving a ++ reaction in the fourth tube. Since these values are approximate the figures have not been extended to further decimal places to express the 25 per cent increase or decrease.

In this way we have assigned values to four degrees of reaction in each tube of the titration as shown in Table IV. This table is used for deriving

unit values from the titration results. The tests recorded in Table II, interpreted in this way, read

For serum I	0	units
For serum II	40	units
For serum III	0.4	units
For serum IV	15	units
For serum V	2.5	units
For serum VI	20	units

TABLE IV

UNIT VALUES ASSIGNED TO REACTIONS OBTAINED BY TITRATION

DEGREE OF REACTION	1	2	3	4	5	6	7	8
++++	0.3	0.6	1.5	3.0	6	12	30	60
+++	0.25	0.5	1.2	2.5	5	10	25	50
++	0.2	0.4	1.0	2.0	4	8	20	40
+	0.1	0.3	0.7	1.5	3	6	15	30

Although only the last reacting tube is used to record the result, the reactions in the preceding tubes should be observed as a control. It was found necessary to make one definite exception to the above method of reading. A few apparently normal sera were found giving slight precipitation in several tubes, such as

Tube	1	2	3	4	5	6	7	8
Reaction	1	1	1	0	0	0	0	0

We have consequently made it a rule to record only the first tube of a series showing a + reaction, and to record such a serum as the above as containing 0.1 unit. A few sera gave reactions in successive tubes, such as 2 2 2 1 0 0 0, and while most of those were from syphilitic cases, we think it safer to record all such atypical reactions as doubtful.

The Kahn units referred to in the following discussion signify values obtained by the above method and not by the original method of Kahn.

CORRELATION OF VALUES OBTAINED BY DIFFERENT METHODS

We have tabulated the results obtained by titration of 907 serums from 433 patients. These specimens were also tested by the routine method of Kahn, by the Wassermann using cholesterin antigen and one hour preliminary incubation at 37°, and by the Wassermann using alcoholic antigen and four hours' incubation in the ice box.⁹ The Sigma reaction was done with 600 of these sera.

It was soon evident that this quantitative Kahn revealed many abnormalities too slight to be detected by the Wassermann method used. Before studying the results obtained it seemed necessary to attempt a correlation of the values obtained by the different methods and to determine what reading in one test is equivalent to a certain reading in another.

The third tube of the standard Kahn test contains antigen and serum in the ratio of 1:12, which is intermediate between the second and third tubes of the titration where the ratios are 1:20 and 1:8 respectively. Consequently, a serum showing 0.4 unit or a ++ reaction in the second tube of the titration should show a slightly weaker, or perhaps the same, reaction in the third tube of the Kahn, and a negative in the second and first tubes. The second and first tubes of the Kahn are intermediate between the third and fourth and the fourth and fifth tubes of the titration. From these considerations the equivalent values shown in Table V have been estimated but it should be remembered that these are only approximate.

The determination of equivalent values for the Wassermann and the quantitative Kahn is more difficult because wider variations in the results were found. This is in accord with general experience, which has shown that the same serum when tested with different antigens will give different values even in parallel Wassermann reactions.

It seemed most useful to take the mean value found in a series of tests. For example, of 40 sera containing 2 units as determined by titration ten gave a negative Wassermann, with cholesterol antigen nine a +, four, a ++, twelve, a +++, and five a ++++. The mean value was ++. From similar titrations the equivalent values shown in Table V were estimated.

TABLE V
EQUIVALENT VALUES FOR KAHN AND WASSERMANN METHODS (APPROXIMATE)

KAHN UNITS	QUALITATIVE KAHN			WASSERMANN	
				CHOLESTERIN ANTIGEN	ALCOHOL ANTIGEN
	FIRST TUBE	SECOND TUBE	THIRD TUBE		
0	0	0	0	0	0
0.4	0	0	+	0	0
1.0	0	+	+++	+	0
2.0	+	+++	++++	++	+
4.0	++++	++++	++++	++++	++++
20	++++	++++	++++		
to	++++	or	++	++++	++++
		++++			
		or			
60	++++	++	0		

TABLE VI
COMPARISON OF VALUES OBTAINED BY KAHN TITRATION AND BY SIGMA REACTION

KAHN UNITS	NO OF SERA	NO SHOWING NEGATIVE SIGMA	LOWEST SIGMA READING	HIGHEST SIGMA READING	AVERAGE SIGMA READING
0	141*	125	0	2.0	0.1
0.4	27	14	0	2.5	0.9
1.0	22	4	0	4.4	1.6
2.0	23†	2	0	5.9	3.4
4.0	30	0	15	21.0	14.0
8.0	39	0	36	114.0	32.0
20.0	24	0	77	244.0	60.0
40.0	7	0	61.0	400.0	229.0

One serum showing Wassermann Reaction +++ and 124 Sigma Units omitted

†One serum showing +++ Wassermann Reaction and 67 Sigma Units omitted

units in the Kahn test and a negative Sigma and Wassermann, and another patient with an aortitis gave only 0.7 units with a ++ Wassermann

TABLE VIII
FINDINGS IN UNTREATED CASES OF SYPHILIS

DIAGNOSIS	NO OF CASES	KAHN TITRATION	SIGMA	CHOISTEIN	WASSERMANN	ALCOHOLIC
Primary	3	15 60	—	++ ++++	++++	++++
Secondary	20	4 50	10 3 311	+++ ++++	+ ++++	++++
Tertiary	25	0 1 60	0 797	0 ++++	0 ++++	++++
Latent	27	15 50	8 3 231	+ ++++	0 ++++	++++

SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

The possibility of determining the significance of weak reactions seemed greater in patients with syphilis of the central nervous system because in the majority of cases the diagnosis can be established by physical signs or by spinal fluid examination without reference to the serum reaction. It is in such cases also that weak and doubtful serum reactions are most frequently found. Most of the patients studied were under treatment, but none of them could be regarded as cured.

Tabulating the results of 233 tests from 50 cases of tabes, 14 cases of meningovascular syphilis and five cases of latent syphilis of the central nervous system (that is cases with positive fluid but no signs of cerebrospinal lesions), the values in the Kahn titration varied from 0.40 units. Twenty-five sera were negative in all tests. 92 gave strong reactions in both Kahn and Wassermann. The results are summarized in Table IX.

TABLE IX
FINDINGS IN SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

KAHN TITRATION			WASSERMANN CHOISTEIN		WASSERMANN ALCOHOLIC	
Negative	0 07	45 sera	0	51 sera	0	70 sera
Doubtful	1 15	29 sera	+	37 sera		
Weak	2 3	55 sera	++	17 sera	+ ++	39 sera
Strong	4 40	104 sera	+++ + +++	127 sera	+++ ++++	123 sera

The advantage of the Kahn appeared in the detection of weakly reacting sera. With these syphilitic specimens it gave more such results than did the Wassermann, whereas with the nonsyphilitic sera (Table VII) the Wassermann gave more weak reactions than did the Kahn.

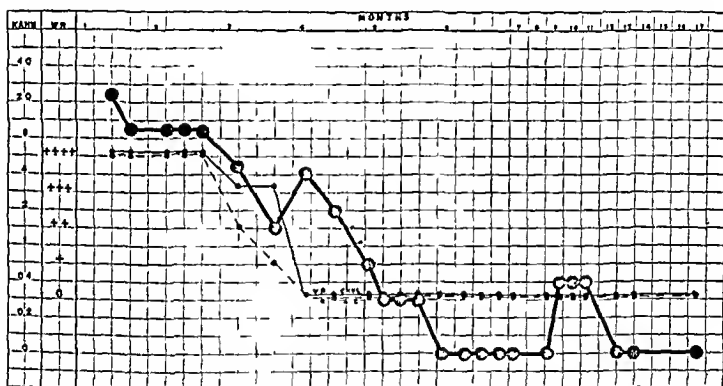
VALUES FOUND IN TREATED CASES OF EARLY SYPHILIS

Further evidence as to the significance of weak Kahn reactions was furnished by tests made on cases of early syphilis during treatment. In most of those which we were able to follow doubtful Kahn reactions persisted after the Wassermann became negative. In some the Kahn also became negative a few weeks after the Wassermann (Chart I), in others a fractional Kahn persisted for a long time before it eventually disappeared (Chart II). These cases

are in contrast with others which were studied after a satisfactory course of treatment early in their infection and which showed for a year or two, titrations which were completely negative except for occasional findings of 0.1 or 0.2 unit

We were able to observe the course of one case in which fractional Kahn reactions appeared of very definite significance. This patient had begun treatment early in the secondary stage. In three months his Wassermann became negative but relapsed to ++++ six months later. The following year the Wassermann again became negative and remained so continuously for two years, except for a single finding of ++ and another of + in the cholestern test. During this period however a weak or doubtful Kahn was almost uniformly found. This gradually increased in strength until in March,

CHART I PERSISTENCE OF KAHN REACTION AFTER WASSERMANN BECAME NEGATIVE



A case of secondary syphilis under treatment. The Wassermann reaction became negative one month before the Kahn became doubtful and two months before it became completely negative. The slight relapse in the Kahn in the ninth to eleventh months followed a period where injections were substituted for injections.

1926, (after three and a half years of treatment) his serum showed five Kahn units and a negative Wassermann. Two months later he returned with a cutaneous relapse evidenced by a hypertrophic papule on the scrotum with a positive Spirochete find a ++++ Wassermann, and a Kahn reaction of 20 units. The reactions for a year preceding the relapse are shown in Chart III.

In this instance it seems that the persistent fractional Kahn reaction was evidence of remaining latent infection. In a number of other cases which had previously shown clinical or serologic relapses, we have found a Kahn of from 0.4 to two units with a negative Wassermann. We have come consequently to regard the repeated finding of so little as 0.4 of a unit as suggestive of persistent infection in treated cases. It is necessary to add that a negative Kahn is no proof of cure as is evidenced by quite negative titrations in cases of known syphilis of the central nervous system.

Comparing the results of the Kahn titration with those of the Sigma reaction, similar wide variations were found. The extent and frequency of those are indicated in Table VI. As the Sigma readings are numerically expressed, it was possible to average them. It will be seen by comparing the figures in the first and last columns that for weaker sera the average number of units estimated was somewhat greater in the Sigma and that for very strong sera the Sigma gave much higher readings.

Two strongly reacting sera which gave a negative or weak Kahn (see Table XI) were omitted from this tabulation because it seemed that these represented definite failures for the Kahn technique and had no bearing on the quantitative relationship of the two methods for numerically representing results.

REACTIONS OBTAINED WITH NONSYPHILITIC SERA

The first point to determine in regard to these reactions was the number of units that might be found by this method in nonsyphilitic sera. This is not a simple problem as one can never rule out the possibility of latent infection. We have, however, tabulated 105 tests from 98 patients who showed no evidence of syphilis. The values found are shown in Table VII.

The cases studied were for the most part patients with diseases of the skin or those referred to a dermatologic clinic on account of some question as to syphilitic infection. The frequency of doubtful Wassermanns was much greater than would be found in the same number of quite normal individuals.

TABLE VII
FINDINGS IN NONSYPHILITIC SERA

QUANTITATIVE KAHN REACTION	WASSERMANN CHOLESTERYL ANTIGEN	WASSERMANN ALCOHOLIC ANTIGEN	SIGMA REACTION (46 SERA TESTED)
0.0 Units 79 Sera	0 91 Sera	0 101 Sera	0 42 Sera
0.1-0.3 Units 13 Sera			
0.4-0.7 Units 9 Sera			1.15 Units 4 Sera
1.5 Units 2 Sera	+		2.2 Units 1 Serum
2.0 Units 1 Serum	++	+	
3.0 Units 1 Serum	+++		15.0 Units 1 Serum

Three other sera from nonsyphilitic patients gave the following readings

Tube	1	2	3	4	5	6	7	8
Serum 1	1	2	1	0	0	0	0	0
Serum 2	2	2	1	0	0	0	0	0
Serum 3	1	1	2	1	0	0	0	0

These were considered as containing less than 0.4 unit on account of the low readings in the first tube. The four sera showing from 1.5 to 3 units were among those which also gave feeble Wassermann reactions.

Reactions in the first tube (0.1-0.3 units) were found so frequently as to indicate that they are of no specific significance. It is hardly possible that all of the 9 patients who showed precipitation in the second tube (0.4 to 0.7 units) had unrecognized latent syphilis. If nonsyphilitic sera may contain

this amount of reacting substance it seems necessary to regard all such reactions as negative except in known syphilitic cases under treatment

Two patients in this apparently normal group showed weak reactions of 2-3 units. One was a case of acne which we have been unable to examine carefully or to follow up. His Kahn titration showed 3 units, his Wassermann with cholesterol antigen +, with alcoholic antigen 0. The other was a case of furunculosis with acetonuria otherwise negative on repeated examination. Spinal fluid was not obtained. Three samples of his serum gave the following results:

DATE	KAHN	SIGMA	WASSERMANN CHOLESTERIN REACTION	WASSERMANN ALCOHOL REACTION
April 21	0 units	2 units	0	0
May 22	0.6 units	1.1 units	0?	0
Nov 10	2.0 units	1.0 units	+	+

Weak reactions of this type are insufficient basis for a diagnosis of syphilis without confirmatory evidence. For that matter no single serum test warrants such a diagnosis. In this small series we have found no Kahn reaction stronger than 3 units in the absence of syphilis but in routine tests with the qualitative Kahn we have met in a few instances with a strongly positive Kahn and a negative Wassermann which was obtained on repetition with the same specimen but not with other specimens from the same patient. The patients were apparently free from syphilis on further examination. The term "positive" implies a finality that these serum tests do not possess. On the basis of these results with non-syphilitic sera we would class our unit findings in the Kahn reaction as follows:

0.0 to 0.7 units	Negative	
1.0 to 1.7 units	Doubtful	Of value only with strongly confirmatory evidence
2.0 to 3.0 units	Weak Reactions	These probably occur occasionally in the absence of syphilis
4.0 or more units	Strong Reactions	Equivalent in significance to a ++++ Wassermann

VALUES IN UNTREATED SYPHILIS

In untreated cases of active syphilis the titration usually showed a high value of from 8 to 60 units. The strength of the reaction bore no relation to the activity of the infection, and very high values were met with in continuously latent cases and in those in which all symptoms had subsided under treatment. One robust man, who gave no history of infection and showed no signs of syphilis on careful examination but whose child had a frank congenital infection, gave a titration of 50 units.

On the other hand low values were occasionally found in patients with definite symptoms. Two who had late secondary exanthems showed only four units by the Kahn reaction, and one of them when tested by the Sigma method showed only 10.3 units.

In tertiary syphilis the values were often higher than in secondary cases. One patient, however, with a definite gumma of the clavicle showed only 1.5

units in the Kahn test and a negative Sigma and Wassermann, and another patient with an aortitis gave only 0.7 units with a ++ Wassermann

TABLE VIII
FINDINGS IN UNTREATED CASES OF SYPHILIS

DIAGNOSIS	NO OF CASES	KAHN		WASSERMANN	
		TITRATION	SIGMA	CHOLESTERIN	ALCOHOLIC
Primary	3	15 60	—	++ +++++	++++
Secondary	20	4 50	10 3 311	+++ +++++	+ +++++
Tertiary	25	0 1 60	0 797	0 +++++	0 +++++
Latent	27	1 5 50	8 3 234	+ +++++	0 +++++

SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

The possibility of determining the significance of weak reactions seemed greater in patients with syphilis of the central nervous system because in the majority of cases the diagnosis can be established by physical signs or by spinal fluid examination without reference to the serum reaction. It is in such cases also that weak and doubtful serum reactions are most frequently found. Most of the patients studied were under treatment, but none of them could be regarded as cured.

Tabulating the results of 233 tests from 50 cases of tabes, 14 cases of meningovascular syphilis, and five cases of latent syphilis of the central nervous system (that is, cases with positive fluid but no signs of cerebrospinal lesions), the values in the Kahn titration varied from 0.40 units. Twenty-five sera were negative in all tests. 92 gave strong reactions in both Kahn and Wassermann. The results are summarized in Table IX.

TABLE IX
FINDINGS IN SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

KAHN TITRATION		WASSERMANN CHOLESTERIN		WASSERMANN ALCOHOLIC	
Negative	0 0.7 45 sera	0	51 sera	0	70 sera
Doubtful	1 1.5 29 sera	+	37 sera		
Weak	2 3 55 sera	++	17 sera	+ ++	39 sera
Strong	4 40 104 sera	+++ +++++	127 sera	+++ +++++	123 sera

The advantage of the Kahn appeared in the detection of weakly reacting sera. With these syphilitic specimens it gave more such results than did the Wassermann, whereas with the nonsyphilitic sera (Table VII) the Wassermann gave more weak reactions than did the Kahn.

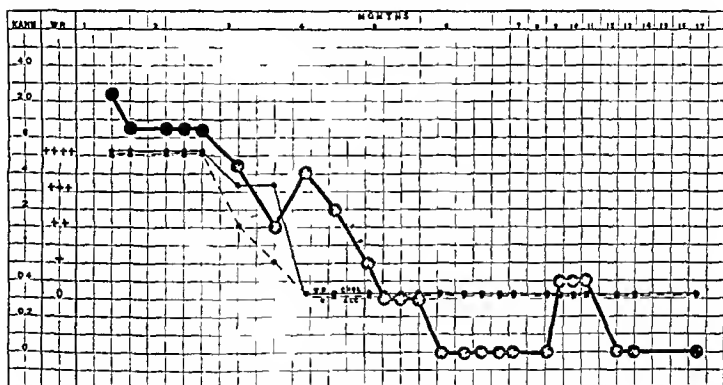
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are in contrast with others which were studied after a satisfactory course of treatment early in their infection and which showed for a year or two, titrations which were completely negative except for occasional findings of 0.1 or 0.2 unit

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A case of secondary syphilis under treatment. The Wassermann reaction became negative one month before the Kahn became doubtful and two months before it became completely negative. The slight relapse in the Kahn in the ninth to eleventh months followed a period where injections were substituted for injections.

1926, (after three and a half years of treatment) his serum showed five Kahn units and a negative Wassermann. Two months later he returned with a cutaneous relapse, evidenced by a hypertrophic papule on the scrotum with a positive Spirochete find, a ++++ Wassermann, and a Kahn reaction of 20 units. The reactions for a year preceding the relapse are shown in Chart III.

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TABLE X

SUMMARY OF DISAGREEMENTS BETWEEN THE WASSERMANN AND THE QUANTITATIVE KAHN REACTIONS

KAHN STRONGER THAN WASSERMANN				WASSERMANN STRONGER THAN KAHN			
KAHN	WASSER MANN	SYPHIL ITIC SERA	NON SYPHIL ITIC SERA	WASSER MANN	KAHN	SYPHIL ITIC SERA	NON SYPHIL ITIC SERA
Strong (4 units)	Negative	6	0	Strong	Negative (0.07 unit)	4	0
Weak (2.3 units)	Negative	25	0	Weak	Negative	13	1
Doubtful (1.15 units)	Negative	5	1	Doubtful	Negative	34	14
Strong (4.8 units)	Doubtful	12	0	Strong	Doubtful	12	0
Weak	Doubtful	43	2	Weak	Doubtful	29	1
		132	3			92	16

ferences in titer seemed significant, however, and we have plotted our curves on abscissae each of which represents one tube in the titration. This is approximately a geometric progression in the number of units.

COMPARISON OF THE KAHN TITRATION WITH THE WASSERMANN

The tabulation of tests made in syphilis of the central nervous system indicated that the quantitative Kahn revealed more positive reactions than the Wassermann. It seemed that a summary of all the instances in which the two methods disagreed might be of interest (Table X). We have grouped the Kahn titrations according to the definitions already given and the Wassermann reactions as follows:

Reactions of ++ with cholesterol antigen and + with alcoholic antigen, or weaker, are classed as doubtful. Reactions of +++ or ++++ with both antigens are classed as strong. All intermediate sera, including these which gave a strong reaction with one antigen and a negative with the other, are classed as weak.

In this summary the Kahn reaction shows to advantage at almost every point of comparison. The most significant item is that of the 14 nonsyphilitic sera which gave a doubtful Wassermann and a negative Kahn. Ten of these showed no precipitation whatever in any tube of the titration, making it clear that the slight inhibition observed in the Wassermann test was not specific.

We have found the Kahn helpful in deciding the often difficult question of the significance of a + Wassermann. The reason for this seems to be that a result in the quantitative Kahn which would be recorded as one unit or a doubtful reaction, is one which shows a weak precipitation (++) in the third tube but a maximum precipitation (++++) in the first tube. This will seldom result from technical error and is never a question of judgment on the part of the reader. On the other hand, a + Wassermann signifies a slight inhibition of hemolysis, which may easily be caused by technical error. The question, moreover, as to whether such a test should be regarded as + or 0 is one to which two readers will frequently disagree, so that a subjective factor enters into the determination of a + Wassermann. The quantitative Kahn method nearly eliminates such errors in recording weak reactions.

There were a few cases in which the Kahn failed. One was that of a patient with a large gumma of the ulna. The first specimen obtained showed an extraordinary prezone, sufficient to mask the qualitative Kahn but not the titration. The third specimen was negative in both the Kahn methods, though still positive in the Wassermann (Table XI).

TABLE XI

SUCCESSIVE TESTS ON A CASE OF GUMMA OF THE ULNA SHOWING AN EXTRAORDINARY PREZONE

DATE	WASSERMANN CHOLESTERIN	ALCOHOLIO	QUALITATIVE KAHN	KAHN TITRATION	SIGMA
9/10	++++	++++	000	0 0 0 0 0 4 0 (30 Units)	311 Units
9/29	++++	++++	+++	3 3 3 3 4 4 3 (50 Units)	153 Units
10/2	++++	++++	000	0 0 0 0 0 0 0	124 Units
10/13	++++	++++	+++++	0 0 2 2 4 4 2 (40 Units)	410 Units

COMPARISON WITH SIGMA REACTION

In the cases studied the values found by the quantitative Kahn method appeared as consistent with the clinical findings as did those by the Sigma. Values of less than one Kahn unit were found in a few normal sera which gave negative Sigma reactions, but reference to Table VII shows that even in a small series of apparently nonsyphilitic cases a positive Sigma reaction of over two units was observed in two instances. It would seem from this that the Sigma is no more nearly specific than the Kahn. Technically, the quantitative estimation by the Sigma method consumed far more time and offered more chances for mistakes. The incubation must be carried on in a water bath carefully controlled as to temperature and depth, and the preparation of the antigen suspension must be performed with extreme care. Errors in either of these steps may interfere seriously with the reaction. We also found the preparation of a satisfactory antigen extract by the Bordet Rouillon method difficult.

We wish to express our indebtedness to Dr H K Ward and Dr P H Jones who kindly helped us acquire the technique of the Sigma reaction and supplied us with the antigen with which the tests here reported were performed.

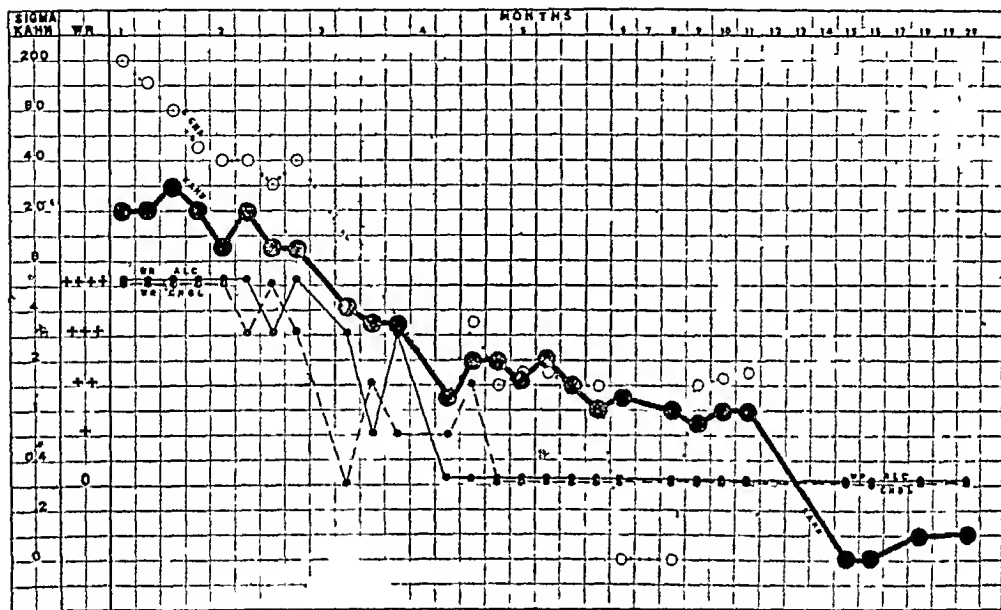
COMPARISON WITH THE QUALITATIVE KAHN

The quantitative method which we have employed differs from the routine Kahn test only in the ratios of antigen and serum employed and in the expression of the results in terms of units. It does, however, indicate the strength of the reaction more clearly than can be done by the qualitative method. In reporting the standard Kahn it is somewhat confusing to give the results obtained in each of the three tubes. On the other hand averaging the results in the three tubes gives a false valuation for many strong sera which show a prezone. We have discussed this question in a previous paper,¹⁰ but the point can be made clear by the following illustration.

QUANTITATIVE ESTIMATION OF STRONG REACTIONS

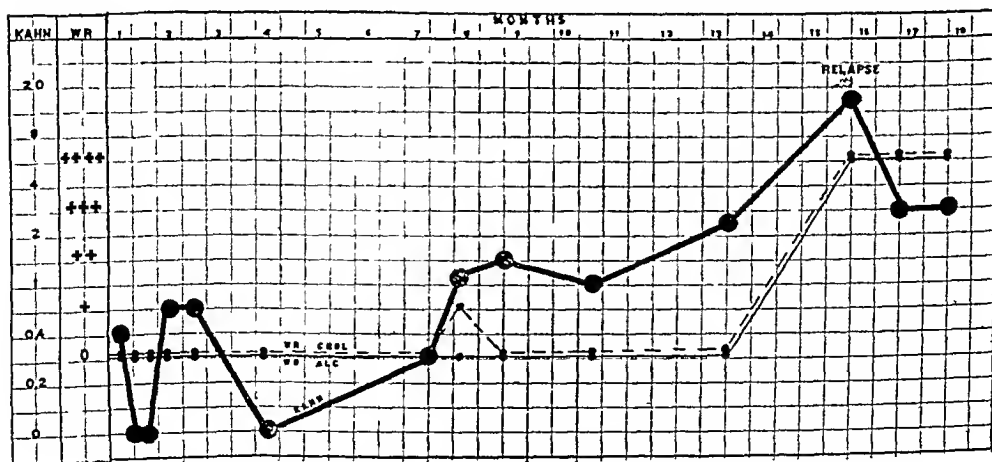
Up to this point we have been concerned chiefly with the interpretation of weak reactions. Another object of these quantitative tests was to follow variations of the reactions in strong sera. In secondary cases a weakening could sometimes be detected before the Wassermann reaction fell below ++++ (Charts I, II, and IV-A), but such a change could more often be ob-

CHART II PERSISTENCE OF KAHN REACTION AFTER WASSERMANN BECAME NEGATIVE



A case of secondary syphilis during treatment. The patient responded slowly. The Wassermann became negative four months after beginning of treatment, the Kahn ten months later. The Kahn showed distinct weakening in the reaction before the Wassermann. The Sigma followed the same general course as the Kahn reaction but its curve showed more irregularities.

CHART III GRADUAL INCREASE IN THE KAHN REACTION PRECEDING A CLINICAL RELAPSE

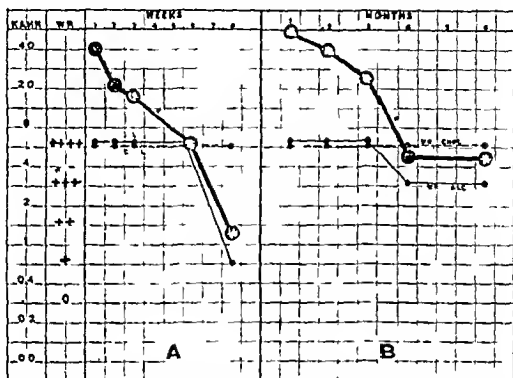


A positive Wassermann was not obtained until after the outbreak of symptoms.

served in late cases which respond more slowly to treatment. In these a gradual weakening of the titration was often the only evidence of response to treatment for a long period during which the Wassermann reaction and qualitative Kahn remained + + + + (Chart IV B)

As to the accuracy of these quantitative determinations, it is difficult to offer proof. The most definite evidence was obtained from cases followed

CHART IV RESPONSE TO TREATMENT SHOWN BY KAHN BUT NOT BY WASSERMANN REACTION

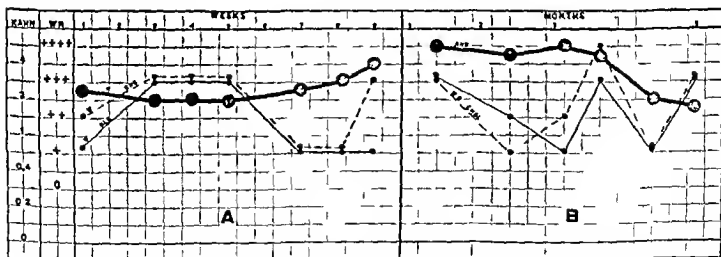


A A case of secondary syphilis during the first two months of treatment.

B A case of tertiary syphilis during the first six months of treatment.

The Kahn showed distinct weakening in the reaction before any change was perceptible in the Wassermann.

CHART V VARIABILITY IN THE WASSERMANN REACTION AS COMPARED WITH THE KAHN



A A case of meningovascular syphilis during two months of treatment. The Kahn showed consistently from 2 to 4 units while the Wassermann varied from + to + + + a year later this patient still showed 5 units in the Kahn test and a + + + Wassermann indicating that the variations in the Wassermann were of no significance.

B A case of late congenital syphilis under treatment. The Kahn showed a slight but consistent weakening in the reaction while the Wassermann showed erratic variations.

during treatment. In general the Kahn showed fewer erratic variations than did the Wassermann as shown in Chart V. Frequently it furnished curves which were more regular than those plotted from the Sigma. Only large dif

In Table XI are shown three sera. The first was from an untreated case of gumma of the leg. The serum gave a Kahn reaction of + + + +, + + +, + + (average + + +). The quantitative test showed 60 units and the other reactions were strongly positive.

The second serum was from a latent case that had been treated off and on for twelve years. The Kahn reaction was + +, + + -, + + + +, which gave the same average as the first specimen, + + +, although the titration showed only two units and the Sigma and the Wassermann indicated a very weakly reacting serum.

The third case was also latent and had been treated with fair thoroughness on the outbreak of the secondary eruption two years before. His qualitative Kahn test was 0, + +, + + + +, which gave an average of + +, but the titration showed only 12 units, and the Sigma and Wassermann reactions were negative.

In these instances reporting the reactions by averaging the three Kahn tubes would have been distinctly misleading, whereas the unit method made clear the difference in strength of the reactions.

TABLE XII

EXAMPLES OF SERA OF WIDELY DIFFERENT STRENGTHS GIVING SIMILAR AVERAGES IN THE QUALITATIVE KAHN

CASE	QUALITATIVE KAHN TUBES				QUALITATIVE KAHN	SIGMA		WASSERMANN	
	1ST	2ND	3RD	AVERAGE				CHOLESTERYN	ALCOHOLIC
Active Tertiary	4	3	2	+ + +	60 Units	465	Units	+ + + +	+ + + +
Treated Tertiary	2	3	4	+ + +	2 Units	24	Units	+	+
Treated Secondary	0	2	4	+ +	12 Units	0	Units	0	0

The choice of an antigen ratio of 1-8 as a basis for unit determination instead of 1-6, as in the qualitative Kahn, is of no importance except that fractions of the amounts used are easier to measure on a standard pipette.

The use of serum-antigen ratios lower than those of the qualitative Kahn enables one to detect weak reactions in sera giving a negative qualitative test and to obtain a maximum degree of precipitation in others which give only a + or + + in the third tube of the standard Kahn. This makes weak reactions more definitely objective. The reactions so determined, though of little use in diagnosis, have seemed of real help in studying cases under treatment.

The employment of higher antigen-serum ratios to differentiate between the strength of sera giving a + + + + with the qualitative test is also of help in observing the effects of treatment.

We now employ tubes two, three, four and five of the titration described in routine work. This enables us to differentiate and report tests of from 0.3 to six units which gives to the clinician a definite indication of the strength of the reaction. The entire titration is set up, when necessary, in cases where it is desirable to observe the effect of treatment.

The method described is not presented as a final one, and it is not expected that exact correspondence in unit values will be obtained by different labora-

ories If this were to be attempted, it would be necessary to introduce a correction factor for each antigen extract used It has seemed, however, sufficiently accurate for comparing strengths of various sera tested in the same laboratory

SUMMARY

1 A simple procedure is described for quantitative estimation of the Kahn reaction This permits a clear expression of the strength of the reaction in terms of arbitrarily defined units

2 In certain patients whose Wassermann remained continuously ++++ under treatment a response to therapy was shown by the diminishing unit value of their Kahn reactions

3 It was also possible in these titrations to detect a persistent abnormality in the sera of many patients whose Wassermann had become negative under treatment While these reactions were too weak to be of diagnostic value, they appeared to be significant when found repeatedly in treated cases

4 When sera which gave a doubtful Wassermann were tested by this method, it was found that nearly all of those from nonsyphilitic patients fell in the negative zone whereas those from cases of late syphilis frequently gave a definite Kahn reaction We believe that the procedure is of real value in interpreting the significance of these doubtful reactions

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LABORATORY METHODS

NOTES ON BASAL METABOLISM*

X SIMPLIFIED DATA BLANK FOR GASOMETER GAS ANALYSIS METHOD†

BY WILLIAM H. STONER, A.M., M.D., PHILADELPHIA, PA.

THE SIMPLIFIED calculation of basal metabolic rate, by the gasometer gas analysis method, described in the preceding note of this series, may be made conveniently on the blank shown in Fig 1, as illustrated by the example given in Fig 2. This blank is so designed as to require a minimum of recording and calculating. It provides for the calculation of basal metabolic rate by the Harris and Benedict, Dreyer and Aub and DuBois standards, respiratory quotient, theoretic or normal weight of Dreyer and all the usual partial values of these calculations.

The numbers in the following description refer to those of Fig 1.

1 The name of the subject is best recorded in the way it is easiest found—last name first, followed by a comma, then first name in full, and finally middle initial. Much time is saved in searching hospital records for old case histories when this method of the United States Government bureaux is used consistently.

2 Varies with the hospital or clinic. In a private hospital this space may be used for room number and in private practice for the patient's address. In research on normals it may be used to designate the group, etc.

3 In recording dates the name or abbreviation of the name of the month is used rather than its number on account of the confusion arising in the minds of European students in the laboratories who have interpreted, for example, 3/6/26 as third of June, 1926. If the number of the month is to be used it should be given the Roman form, III/6/26, or better 6/III/26.

4 Case number, hospital serial number or group number may be recorded here as is found convenient.

*From the Biochemistry Laboratory and the Department of Metabolic Diseases of the Graduate School of Medicine of the University of Pennsylvania.

Received for publication March 15 1927.

†Preceding Notes of this series appeared as follows:

- I Modified Clinical Method of Determination Boston Med and Surg Jour 1923 clxxxix 193
- II A Simplified Data Card for Clinical Determination Boston Med and Surg Jour 1923 clxxxix 195
- III Errors of Clinical Determination Boston Med and Surg Jour 1923 clxxxix 232
- IV Selection of Normal Standards Boston Med and Surg Jour 1923 clxxxix 236
- V Tables of Values for Dreyer's Formulas Boston Med and Surg Jour 1923 clxxxix 239
- VI Complementary Tables of Values of Dreyer's Formulas Boston Med and Surg Jour 1924 cxci 1026
- VII Actual versus Theoretic Weight in Dreyer's Formulas Boston Med and Surg Jour 1924 cxci 1030
- VIII Tables of Values of the DuBois Surface Area Formula JOUR LAB AND CLIN MED 1926 xi 355
- IX Simplified Calculation for Gasometer Gas Analysis Method JOUR LAB AND CLIN MED 1927 xii 884

5 The possibility of error in recording sex of a patient was discussed in No III of this series of notes

6 Nude weight in pounds Many hospitals are not equipped with scales weighing in kilograms If the subject is weighed in kilograms, this space is left blank

7 Nude weight in kilograms is obtained either by direct weighing, or

GRADUATE SCHOOL OF MEDICINE—UNIVERSITY OF PENNSYLVANIA
BASAL METABOLIC RATE

Name — 1				Wed — 2				D — 3				Th — 4			
Sex — 5				Weight — 6				lbs. 156 — 7				kg			
Age — 8				Stature — 9				ins 74 — 10				cm			
Sitting height — 14				cm				W ₀ — 16				S & A is to — 12			
Chest circum. — 15				m				W — 11				S m — 13 = 11			
				S m — 18				- 2 = 19				= 16			
Diagnosis —				Baromet — 20				-(21 + 22) = 23				mp			
Pulse — 23				Ref e — 23				On p — 24				C last — 0.93			
Respiration — 24				— 24				Final read g — 25				30			
Not — 25				Initial adj g — 26				Diff — 27				22 = 23			
				C g d mpe re — 31				273 = 32				= 1			
				G A b											
B 1 R ding C 1 1 m				34 27 38				CO ₂ — 41							
								CO — 42							
Appar at — 39				Analyt — 40				Ave gr CU — 43				- 04 48 — 49			
								A rag CO ₂ — 44							
No 2 R adi g Cor 1 1 m				36 27 38				S m — 45							
								K — 46				2481 - 0 = 47			
												50			
								CO ₂ — 41							
								CO — 42							
Appar at — 39				A ly t — 40											
								B M R (H ref nd B edict) = $\frac{P}{K} \times \frac{1}{1.1}$				= 100 = 31			
Dm — 32								B M R (Dy re) = $\frac{(100) H}{D}$				= 100 = 53			
A — 34								B M R (A b nd Du Buf) = $\frac{(100) H}{24 A}$				= 100 = 56			
S — 35															

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Fig 1—Simplified data blank for determination of basal metabolic rate by the gasometer gas analysis method The numbers correspond to paragraph numbers in the descriptive matter

from the weight in pounds by slide rule or calculating machine multiplication by 0.454, or, better, by reference to a conversion table or scale such as is found in many engineering handbooks, physical tables and laboratory manuals Convenient conversion scales are given by Carpenter¹ and Joslin² Sanborn³ gives a conversion table

8 The age is recorded in years to the nearest year

9 Standing height, without shoes or slippers, in inches If stature is measured in centimeters, this space is left blank

10 Stature in centimeters may be obtained either by direct measurement or by multiplication by slide rule or calculating machine of the stature in inches by 2.54 A conversion table³ or scale^{1, 2} is a convenience

11 Weight factor is the numerical value of $66.473 + 13.572 w$ (males)

GRADUATE SCHOOL OF MEDICINE—UNIVERSITY OF PENNSYLVANIA

BASAL METABOLIC RATE

Name—*Jones, Miss Mary A* Ward—*Physiology 15* Date—*20/X/26* No—*P-468*

Sex—*F* Weight—*130 1/4* lbs $\times 454 =$ *59.2* kg W factor—*12.21*

Age—*25* Stature—*63 3/4* ins $\times 2.54 =$ *162* cm S & A factor—*183*

Sitting height—*57 0* cm W₁—*60.6* Sum—*1404 = 11*

Chest circum—*72.5* cm W₂—*52.5*

Sum—*1131 + 2 = 56.5 = W*

Diagnosis—*Normal* Barometer—*775.6* $-(46 \times 3.9) = 745.4 = p$

Pulse — before — During — After — 70 Gasometer—*P-1* Constant—*0.13 = h*

Respiration—*20* — *20* — *20* Final reading—*42.6*

Notes—*BMR = -6 15/X/26* Initial reading—*3*

Diff—*42.3 - 3 = 39.3 = 56.6 = v*

Centigrade temperature—*27* $+ 273 = 300 = t$

Gas Analysis

No 1 Readings Corr Volume

9975 0 *9975* CO—*318*

9460 *+3* *9457* O—*1732*

9455 *+3* *9458*

7930 *+5* *7935*

Average CO—*319 - 0 = 315* *84 = P O*

Apparatus—*H-1* Analyst—*S* Average O—*1732*

Sum—*20.51*

No 2 Readings Corr Volume

9770 *+1* *9771* $\frac{1}{2}$ CO—*319*

9475 *+3* *9478* O—*1731*

7775 *+5* *7780*

Apparatus—*B-2* Analyst—*R*

BMR (Harris and Benedict) = $\frac{p}{k} \frac{c}{t} \frac{1}{H} - 100 = -6.2$

D = *1373* BMR (Dreyer) = $\frac{(r + 100) H}{D} - 100 = -3$

A = *37.0* BMR (Abb and DuBois) = $\frac{(r + 100) H}{4.5 \sqrt{A}} - 100 = -8$

S = *162.9*

Fig 2—Simplified blank showing data of an actual determination

or $655.096 + 9.563 w$ (females), in which w is the nude weight in kilograms. Values of the weight factor for male and female adults are tabulated by Harris and Benedict,⁴ Carpenter,¹ Benedict,⁵ and Joslin.²

12 Stature and age factor is the numerical value of $5.003 s - 6.755 a$ (males) or $1.850 s - 4.676 a$ (females), in which s is stature in centimeters and a is age in years. Values of this factor for adult males and females are tabulated ^{1, 2, 4, 5}

13 The sum of the weight factor and the stature and age factor is expected daily heat production in calories according to the prediction formulas of Harris and Benedict.⁴ This space may also be used for recording the expected twenty four hour calorie production in children according to the standards of Benedict and Talbot,⁶ or of Benedict.⁷

14 Stem or trunk length in centimeters according to the method of measurement of Dreyer and Hanson.⁸

"It is taken with the subject sitting on the platform the following points being carefully observed

"The subject places the backs of the fingers upon the platform on which he sits, and, with the fingers pointing backwards and the knees flexed, lifts the lower portion of the body gently backwards until the lowest bony portion of the os sacrum is in contact with the front of the measuring standard. The back is then straightened until the back of the head comes into contact with the standard. It will be found that different persons require to bend the knees in different degrees in order to achieve this position. The head should be tilted neither up nor down and the eyes should look straight forward. The measurement thus obtained gives the distance between the ischial tuberosities and the top of the head.

"If no proper measuring stand is available fairly accurate readings can be obtained in the following manner. The subject should be seated on a level floor or a board, with his back against the perpendicular projecting angle of a wall or cupboard to which the scale is fixed. He should then proceed to seat himself in the manner indicated above. On account of the influence of the gluteal muscles, the trunk length should not be taken when the subject is seated in a chair, as this affords measurements that are inconstant and that have been found to be as much as 3 per cent greater than those taken by the correct procedure."

15 Chest circumference in centimeters measured as follows.⁹

"The circumference of the chest should be measured by a tape measure in direct contact with the skin (or if necessary placed over a very thin garment). The measurement is taken at the nipple level in males, that is to say, at the level of the fourth intercostal space in the nipple line, in the case of females the measure is taken at the same level just under the breasts. If the measurement in the case of females is taken at the same level over the breasts it is found to be on the average $4\frac{1}{2}$ per cent greater than if taken below the breasts. It is therefore necessary to subtract $4\frac{1}{2}$ per cent from the chest measurement taken in this way, or, as an alternative method multiply the observed measurement by 0.957 before looking up the corresponding weight in the tables.

"While being measured, the subject should stand up with the arms hanging loosely at the sides, and should be encouraged to talk, in this way quiet, natural breathing is secured and expansion of the chest beyond the resting position is prevented. The measurement required is that of the normally breathing, not expanded chest."

16 Theoretic weight in kilograms based upon trunk length according to the formulas

$$W_1 = {}^{0.319} \sqrt{0.38025 \lambda} \text{ (males) or } {}^{0.313} \sqrt{0.36093 \lambda} \text{ (females)}$$

in which W_1 is theoretic weight in grams and λ is sitting height in centimeters. Values of these formulas for the usual sitting heights of males and females are tabulated in No. VII of this series of notes.

17 Theoretic weight in kilograms based upon chest circumference according to the formulas

$$W_{ch} = {}^{0.365} \sqrt{0.662 ch} \text{ (males) or } {}^{0.284} \sqrt{0.30213 ch} \text{ (females)}$$

in which W_{ch} is theoretic weight in grams and ch is chest circumference in centimeters. Values of these formulas for the usual chest circumferences of males and females are tabulated in No. VII of this series of notes.

18 Sum of Nos. 16 and 17 for the purpose of obtaining the arithmetic mean

19 Average of Nos. 16 and 17 or theoretic weight based upon sitting height and chest circumference according to Dreyer and Hanson.⁸

20 Barometer reading in millimeters of mercury. If this reading is recorded in inches and the corrections 21 and 22 are made in inches, the constant 0.0193 must be changed to 0.000761.

21 Tension, in millimeters, of aqueous vapor at temperature, t . Values of the tension of aqueous vapor are tabulated in numerous textbooks of physics and chemistry, in engineering handbooks and by Carpenter,¹ and Hawk and Bergem.⁹

22 Correction in millimeters of reading of mercurial barometer with brass scale for temperature. If this brass scale barometer temperature correction is ignored, an error of less than 0.5 per cent is introduced. Tabular values of this correction are given by Hawk and Bergem,⁹ Carpenter,¹ Sanborn³ and by various physico-chemical tables.

23 Corrected pressure in millimeters of mercury under which the expired air is measured.

24 Identification letter or number of gasometer used.

25 Reading on gasometer scale in centimeters at end of ten-minute test after valves are closed.

26 Reading on gasometer scale in centimeters at beginning of ten-minute test before valve is opened to permit subject's expired air to enter gasometer.

27 Difference between Nos. 25 and 26, which is the height the gasometer bell rose during a ten-minute test.

28 Gasometer factor, or one one-thousandth of the area of the inside cross-section of the bell in square centimeters, determined as described by Boothby and Sandiford¹⁰ or by Hawk and Bergem.⁹

29 Volume in liters at observed temperature and pressure, of the ten-minute specimen of expired air, obtained by multiplying 27 by 28. In laboratories having but one or two gasometers this multiplication may be obviated by dividing the constant 0.0193 by the gasometer factor, using the quotient so obtained instead of the constant 0.0193 and using simply the

height in centimeters rise of the gasometer bell, 27, for v in the final formula. This eliminates one multiplication from each determination and is recommended where a series of determinations are made on one subject with the same gasometer as in the seven or more determinations in respiratory quotient curve studies before and after dextrose administration.¹¹

30 The constant 0.0193 is simply a collection of the constants of a determination of basal metabolic rate: 273 (normal absolute temperature) multiplied by 144 (the number of ten minute periods in twenty four hours), divided by 760 (normal pressure in millimeters of mercury) equals 51.73, the reciprocal of which is 0.0193. Various values may be given to this constant. If, for example, the Aub and DuBois standards alone are used, 24 times 0.0193 equals 0.464 is substituted for 0.0193. The final formula to be used in this case is given in the preceding note of this series. Again, as stated in paragraph 20, if pressure is expressed in inches rather than millimeters, the constant becomes 0.000761. The constant is changed to 0.00193 if the length of the test is not ten minutes, and in this case the time factor in minutes is introduced into the denominator of the final formula. As there appears to be no good reason, however, for not making the duration of the test ten minutes, elimination of the time factor calculation is recommended.

31 Temperature in degrees centigrade of the expired air in the gasometer at the time of final measurement.

32 For the purpose of the final formula the temperature is expressed in absolute degrees so that the gas volume may be corrected for temperature by a simple slide rule multiplication rather than by a tabular factor or by the usual factor $1/(1 + 0.003665 t)$.

33 Record of the pulse rate in beats per minute before, during, and after the test.

34 Record of the respiration rate in respiratory cycles per minute before, during, and after the test.

35 Notes bearing upon the determination such as extent of cooperation, excitement or movement of subject, dates and results of previous determinations, general satisfactory nature of test, etc.

36 Actual burette readings of the duplicate gas analyses, as they are made. All the readings are recorded, and when constant volumes are obtained upon complete absorption of CO and O_2 the readings are simply checked.

37 The corrections are understood to be all in the thousandths place, although the decimal point and ciphers are omitted.

38 Corrected relative volumes of sample, sample minus CO_2 , and sample minus $(\text{CO}_2 + \text{O}_2)$.

39 Identification letter and number of analysis apparatus. In these laboratories, where for teaching purposes many modifications of the original Hal dane apparatus are employed, letters distinguish the various modifications. He for Henderson modification, Ba for Bailey, Bo for Boothby, N for Newcomer, Ha for Harris, etc.

40 Initials of analysts. When possible the duplicate analyses should be performed by different analysts and with different apparatus.

41 The percentage of CO_2 is most simply calculated on a commercial calculating machine by subtracting from the corrected relative volume of the sample the corrected relative volume for absorption of CO_2 and dividing the remainder by the corrected relative volume of the sample. Decimal points may be ignored.

42 The percentage of O_2 is calculated similarly by subtracting from the corrected relative volume after absorption of CO_2 the corrected relative volume after absorption of O_2 and dividing the remainder by the corrected relative volume of the sample.

43 The average of the two CO_2 determinations, which should be rejected if at variance by more than 0.02 per cent.

44 The average of the two O_2 determinations, which should be rejected if at variance by more than 0.03 per cent.

45 The sum of the CO_2 per cent and O_2 per cent of the expired air.

46 The nitrogen percentage of the expired air may be set down by inspection by subtracting No. 45 ($\text{CO}_2 + \text{O}_2$) from 99.99.

47 The nitrogen percentage of expired air is multiplied (best by a calculating machine) by 0.2648 and from the product, without recording on paper, is subtracted the average oxygen percentage, 44. This factor is the denominator of the formula for respiratory quotient and also appears in the final formula for basal metabolic rate. See preceding notes of this series.

48 The difference between the average percentage of CO_2 in the expired air and 0.04 is the numerator of the respiratory quotient.

49 No. 48 divided by No. 47 gives the respiratory quotient.

50 Colorific value of one liter of oxygen at normal temperature and pressure according to the table of Zuntz and Sehumburg¹² or of Magnus-Levy.¹³ See preceding note of this series. The former tables are transcribed by Boothby and Sandiford,¹⁰ Carpenter,¹ Sanborn,³ and Hawk and Bergeim.⁹

51 Basal metabolic rate by any method based upon 24 hour standard calorie production, as Harris and Benedict,⁴ Benedict and Talbot,⁶ or Benedict.⁷ The method of calculation of the formula was discussed in the preceding note of this series.

52 Calorie production per day according to the formulas of Dreyer.¹⁴ Tabular values of these formulas appear in Nos. V and VI of this series of notes.

53 Basal metabolic rate according to Dreyer's standards. If only the Dreyer result is desired, the formula for the Harris and Benedict basal metabolic rate is used substituting D for H . If, however, the Harris and Benedict result has been calculated, the Dreyer result is most simply obtained by multiplying (by slide rule) the value of the fractional part of the Harris and Benedict formula by H and dividing by D as indicated in the formula.

54 Calorie production per hour per square meter body surface according to the normal standards of Aub and DuBois.¹⁵ These values are tabulated by Boothby and Sandiford,¹⁰ Carpenter,¹ Benedict,⁵ Sanborn,³ and in No. IV of this series of notes.

55 Body surface area in square meters according to the formula of

DuBois and DuBois¹ Graphs of the values of this formula have been published by Boothby and Sandiford,¹⁰ Carpenter,¹ Benedict, Sanborn² and tabular values appear in Sanborn¹ and more fully in No VIII of this series of notes

56 Basal metabolic rate according to the normal standards of Aub and DuBois If only the Aub and DuBois result is desired the formula as stated for the Harris and Benedict basal metabolic rate is used substituting 0.464 for A , and S times A for H (See preceding note of this series) If however, the Harris and Benedict result has been determined, the Aub and DuBois result is most simply obtained by multiplying (by slide rule) the value of the fractional part of the Harris and Benedict formula by H and dividing by 24, by S and by A , as indicated in the formula

SUMMARY

There is described and illustrated a blank for recording the data and calculations, without logarithms, of the determinations, by the gasometer gas analysis method, of respiratory quotient and of basal metabolic rate by the standards of Harris and Benedict of Dreyer and of Aub and DuBois

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THE ESTIMATION OF PLASMA CHLORIDES*

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SINCE the publication by Volhard in 1874 of his simple method for the estimation of chlorine in an acid solution, many variations of the procedure have been described, and in many of these the method has been used for the estimation of chlorides in blood plasma. The advantages and the disadvantages of the various procedures have been fully discussed in papers such as these by Rappleye, Greenwald and Weiss, Whitehorn, and Van Slyke and his coworkers. It has seemed to us that in many instances the simple procedure as described by Volhard has been made unnecessarily complex and time consuming. The method of McLean and Van Slyke necessitates two filtrations whereas the remainder of the methods in current use, as far as we know, necessitate one filtration. This, of course, presupposes accurate dilutions and the use of definite aliquot portions. Any method by which filtrations and accurate dilutions can be avoided is of considerable advantage in large clinical laboratories in which many chloride estimations must be carried out with sufficient accuracy for clinical interpretation. For several years during which we made a great many determinations of chlorides in solutions containing precipitates of organic materials, and in the presence of the acid color of such dyes as phenolsulphonephthalein, we have had no difficulty in accurately determining volumetrically the chloride content of the solutions. In other words the end point as shown with ferric ammonium sulphate as indicator in the presence of other colors is sharp and distinguishable, and no loss of chlorides has taken place by reason of the presence of various precipitates.

With knowledge of the foregoing it seemed entirely possible that with very little experience one should be able to determine the chlorides in blood plasma in the presence of a protein precipitate, silver chloride, and the yellow of iron oxalate. Accordingly, we carried out a series of parallel determinations by the method of Whitehorn and the method described further on. These determinations were made entirely on blood plasma from patients and included the estimation of definite added amounts of sodium chloride as well as the determination of the chloride content. In the titrations by the method described we have used as an end point the first change in color that spreads throughout the whole solution. This end point obviously is transient and differs, for instance, from that of Whitehorn who uses a color that is permanent for fifteen seconds. The use of the first change in color which spreads throughout the whole solution is perhaps open to adverse criticism theoretically, but for practical purposes it gives a high degree of accuracy.

*From the Section on Biochemistry Mayo Clinic Rochester Minnesota
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and we feel that it is more easily reproducible and less confusing than one which involves a time interval. This is shown by the quantitative recovery of chlorides added to blood plasma (Table I)

TABLE I

RECOVERY OF SODIUM CHLORIDE AFTER ITS ADDITION TO 1 CC OF OXALATED HUMAN BLOOD PLASMA

SAMPLE	MILLIGRAMS SODIUM CHLORIDE ADDED	MILLIGRAMS SODIUM CHLORIDE FOUND	MILLIGRAMS SODIUM CHLORIDE RECOVERED
AUTHOR'S METHOD			
0	0.00	5.78	0.00
1	0.24	6.02	0.24
2	0.48	6.27	0.49
3	0.72	6.56	0.72
4	0.96	6.76	0.98
5	1.20	6.98	1.20
6	1.44	7.22	1.44
7	1.68	7.47	1.69
WHITEHORN METHOD			
0	0.00	5.80	0.00
1	0.24	6.09	0.24
2	0.48	6.33	0.48
3	0.72	6.57	0.72
4	0.96	6.81	0.96

METHOD

The method which we recommend and which we have used in hundreds of estimations, checked in duplicate by the method of Whitehorn, is as follows. One cubic centimeter of plasma is pipetted into a 125 cc Erlenmeyer flask and to this is added 10 cc of 1.3 nitric acid. The nitric acid is added preferably from a burette slowly and with agitation of the blood plasma so that a white, flocculent precipitate of the plasma proteins is obtained. If the nitric acid is poured in rapidly a curdy and rather sticky precipitate is formed which may occlude a certain amount of chlorides within the curds. To this suspension is added 5 cc of N/35.46 silver nitrate solution, then 1 cc of a 20 per cent ferric ammonium sulphate solution. The excess silver is back titrated with N/35.46 ammonium or potassium thiocyanate. These solutions are of the same normality as that used in the Whitehorn method, hence the calculation of results is the same as he has described. One cubic centimeter of the silver solution is equivalent to 1 mg of chlorine, or 1.65 mg of sodium chloride. We use as the end point as mentioned the first trace of color which spreads throughout the whole solution from the point at which the drop strikes while the flask is gently rotated. This color, of course, fades within a few seconds, but technicians have never experienced any difficulty in perceiving the change, and we believe that this end point is more reproducible than an end point such as is described by Whitehorn when a definite interval of time is prescribed.

The results obtained by adding known amounts of sodium chloride to oxalated blood plasma are shown in the tabulation. It may be seen that the recovery is quantitative in every respect and is sufficiently accurate for

research studies as well as for clinical work in which such a high degree of accuracy is not necessary. The procedure was carried out with like accuracy of recovery by the method of Whitehorn, but to some technicians the end point in this method is more confusing and it is only after many determinations that they are able to check their results. This, however, is not an argument against the method and we have employed it with satisfactory results.

In Figs 1 and 2 are incorporated the results obtained in 100 representative determinations carried out in parallel by the method described herein and by that described by Whitehorn. The values obtained by the Whitehorn method are given in Fig 1 and the deviations from these values, as obtained by our method, are given in Fig 2. The values check closely and in all instances agree within 17 mg of sodium chloride for each 100 cc of blood plasma. Fifty-nine per cent of the determinations checked exactly, 11 per

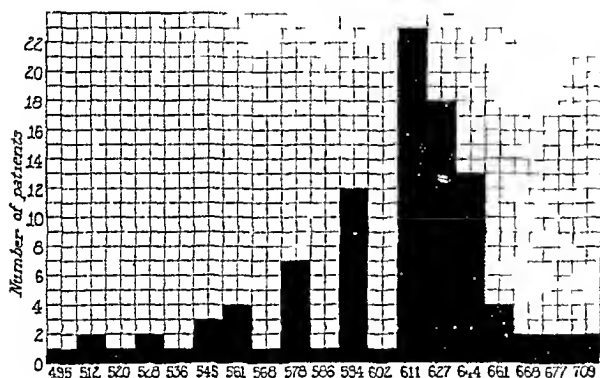


Fig 1

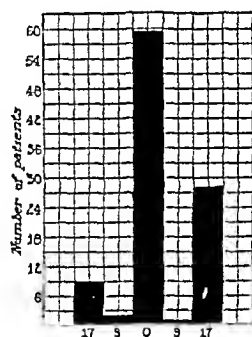


Fig 2

Fig 1—Distribution of plasma chloride values in 100 cases by the Whitehorn method.

Fig 2—Distribution of the difference value between the proposed method and that of Whitehorn in the 100 cases of Fig 1.

cent gave values up to 17 mg per cent lower, and 18 per cent of the series gave values up to 17 mg per cent higher than that obtained by the Whitehorn method. This means that the titrations by the two methods have all agreed within ± 0.1 c c.

SUMMARY AND CONCLUSION

Our results have convinced us that it is unnecessary to remove by filtration either the plasma protein precipitate or the silver chloride precipitate in order to secure highly accurate results by the Volhard titration method as applied to oxalated blood plasma. The red ferric thiocyanate is readily distinguished in the presence of the lemon yellow iron oxalate, as well as in the presence of the protein and silver chloride precipitates. No adsorption of sodium chloride on these precipitates which interfered with the recovery of sodium chloride in blood plasma, was experienced. The red iron thiocyanate color is transient for reasons discussed in previous papers on this subject, but we believe that with the employment of the first trace of excess thiocyanate

throughout the solution a correct and reproducible end point is obtained. This method does not necessitate accurate dilutions, filtrations, or the pipetting of an aliquot portion. The only steps which necessitate quantitative technique are the pipetting of the sample, the addition of a definite quantity of silver nitrate, and a quantitative back titration with a standard solution of potassium thiocyanate. Accurate estimation of blood plasma chlorides may be made on 1 cc of oxalated blood plasma directly by the Volhard titration without the removal of plasma proteins or precipitated silver chloride.

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SOME USEFUL MODIFICATIONS OF THE HALDANE GAS ANALYSIS APPARATUS* †

By VINCENT DU VIGNEAUD B S M S ROCHESTER NEW YORK

FOR some work which was being planned in this laboratory, a gas analyzing machine of greater accuracy than the ordinary Haldane apparatus was desired. Carpenter¹ has designed a burette of 40 cc capacity of sufficient accuracy, but the use of his machine is confined to the analysis of air containing less than 17 per cent carbon dioxide. For the purpose in hand it was necessary to design one that could be used for the determination of respired air and was applicable to the Tissot method of determining metabolism where the percentages of carbon dioxide are usually above 17 per cent. Since for accuracy the readings must be in capillaries, the length of the burette becomes an important consideration if a large capacity is desired.

A burette was required which could be used for the analysis of air containing carbon dioxide in percentages up to 0.4 per cent and from 21 to 50 per cent. In order to allow for such carbon dioxide percentages and to keep the length of the burette within reasonable limits 2 bulbs were blown on the burette besides the usual bulb for holding the nitrogen residual. These bulbs were placed in such positions that one provided for the major portion of the change in volume due to the absorption of oxygen as in the Carpenter mod-

*From the Department of Vital Economics University of Rochester

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†The glass parts were made by the Technical Glass Company of Rochester New York to whom the author is indebted for their excellent work and willing cooperation

fication, and the other took care of a large part of the change in volume due to absorption of carbon dioxide. The latter was so placed that it would lie between the range of most carbon dioxide percentages in outside or room air and the range of most percentages in expired air.

Following Carpenter's suggestion, the divisions of the burette are marked in percentages of 40 c.c. rather than in actual cubic centimeters. Thus the total capacity of the burette from *C* (Fig. 1) to the lowest graduation is 40.08 c.c., and this lowest graduation is marked 100.20. The size of the capillary was so chosen that 1 cm. in length has approximately a capacity of 0.04 c.c. representing about 0.1 per cent of the total volume. This distance being divided by ten graduations giving divisions differing by 0.01 per cent, 1 mm. apart, 0.001 per cent can easily be estimated.

The capillary, *ab*, allows for the reading of the original volume of gas and for the determination of small percentages of carbon dioxide. For convenience graduations above 100.0 per cent can be inscribed, and the range of the low carbon dioxide percentages can be increased considerably. The present burette is calibrated to 100.2 per cent allowing for the analysis of carbon dioxide up to 0.4 per cent.

The capillary, *bc*, is graduated from 2.3 to 5.0 per cent, and in this capillary is measured the volume of oxygen and nitrogen after the absorption of the carbon dioxide. If low carbon dioxide content is expected, one can start with an original volume close to 99.8 per cent, and samples containing as low as 2.1 per cent carbon dioxide can be analyzed.

The bulb, *c*, allows for the absorption of oxygen, and thus the reading of the residual nitrogen falls in capillary, *cd*. The range of this capillary depends on two factors, the respiratory quotient and the carbon dioxide content of the gas. If the *R.Q.* is unity, the percentage of the nitrogen of the expired air will be equal to that of the inspired air. If the latter is outdoor air this percentage of course will be 79.03 per cent. As the *R.Q.* grows smaller than 1, the percentage of nitrogen in the expired air will grow larger. On the other hand, as the *R.Q.* becomes greater than 1, the percentage of nitrogen will become less than the nitrogen percentage of outdoor air. For a given carbon dioxide content the highest *R.Q.* expected will therefore determine the lowest graduation to which the capillary must extend, while the lowest *R.Q.* expected will determine the upper limit.

As the percentage of carbon dioxide increases, the difference between the percentage of nitrogen in the expired air and that of the outdoor air increases. For a given range in the change of the *R.Q.*, the maximum carbon dioxide percentage expected will therefore determine the upper and lower limits which this capillary must have.

The following equations have been developed for the purpose of calculating the maximum and minimum percentages to which the capillary must be graduated for a given range of *R.Q.* and for a given maximum of carbon dioxide content.

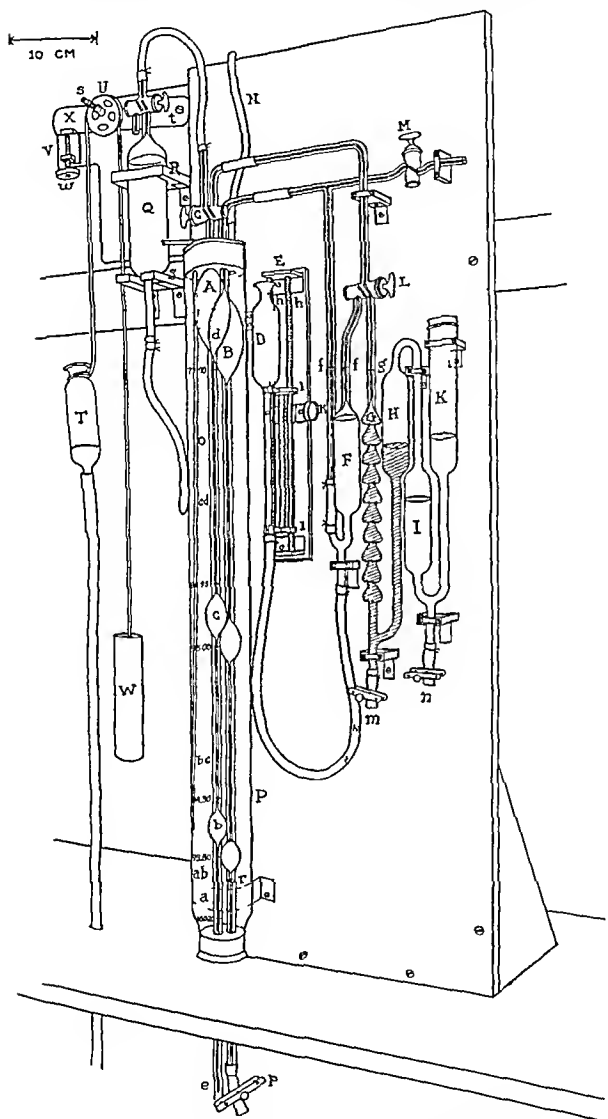


Fig 1—Diagram of the modified Haldane gas analysis apparatus

$$1 \quad R \quad Q = \frac{C'V' - C V}{O V - O'V'} \text{ and } V = \frac{N'}{N} V'$$

C is the per cent CO in inspired air
 O is the per cent O in inspired air, where
 N is the per cent N in inspired air
 V is the volume of inspired air
 C' is the per cent CO_2 in expired air
 O' is the per cent O in expired air
 N' is the per cent N_2 in expired air
 V' is the volume of expired air

By substituting $\frac{N'}{N}V'$ for V , we get

$$2 \quad R \quad Q = \frac{C N - C' N'}{O N' - O' N},$$

but $O = 100 - C - N$, and $O' = 100 - C' - N'$,
therefore

$$3 \quad R \quad Q = \frac{C N - C' N'}{100 N' - N' C - N' N - 100 N + N N'}$$

By rearranging,

$$4 \quad N' = \frac{N (C' + 100 R Q - C' R Q)}{100 R Q - C R Q + C}$$

For outdoor air $N = 79.03$

$C = .04$

$O = 20.93$

Substituting these values in equation 4, we have

$$5 \quad N' = \frac{79.03 (C' + 100 R Q - C' R Q)}{100 R Q - .04 R Q + .04}$$

$$6 \quad \text{or } N' = \frac{0.79062 (C' + 100 R Q - C' R Q)}{R Q + 0.0004}$$

For the present burette $C' = 5$ per cent, and the desired $R Q$ range is from 0.67 to

14 Substituting these values in equation No 6 we would get,

when $R Q = 14$, $N' = 77.91$,

and when $R Q = 0.67$, $N' = 80.96$

For this burette therefore the capillary, cd , must be graduated between 77.91 and 80.96. With the bore selected it would have a length of about 30 cm. From the above equations the maximum and minimum nitrogen percentages can easily be calculated for the design of a burette that will be applicable over the range one wishes. Within a certain length of burette as a limit, a greater carbon dioxide capacity can be allowed for if the variation in $R Q$ is within narrower limits. In the present burette, if we had allowed for a variation of only 0.7 to 1.0, the length of this capillary (cd) could have been shortened eleven centimeters.

With a length of 30 cm for cd , 21 cm for bc , and 3.5 cm for ab , and allowing 15.5 cm for bulb A plus the stem to the stopcock C , 5 cm for bulb c , and 2 cm for bulb d , the length of the burette from stopcock C to the 100.20 per cent graduation is 77 cm. As shown in Fig 1, the burette was placed on the table so that the burette passes through the table and is connected to the mercury leveling bulb by means of rubber tubing below the table. When the burette is made, a one-way stopcock is fused to the end, e , for use in calibration and is removed when the latter is completed. The compensating tube, B , is made similar in form to the measuring burette and of approximately the same volume.

For convenience and accuracy of setting the levels f, f , the HH reservoir, D is raised and lowered by means of a rack and pinion as shown in Fig 1. The rack l is fastened to the two carriages i, i which slide along the two brass rods, h, h .

The oxygen absorber is of the Krogh type as used by Carpenter. Bulbs I and K are used for the purpose of erecting a water seal to protect the pyrogallol from contact with the outside air. The petcocks m and n , are used in filling and emptying the pyrosystem as explained by Carpenter.

The burettes are immersed in a water bath P , which is stirred by a slow stream of bubbles from the inlet tube O , by means of suction applied at N from a water pump.

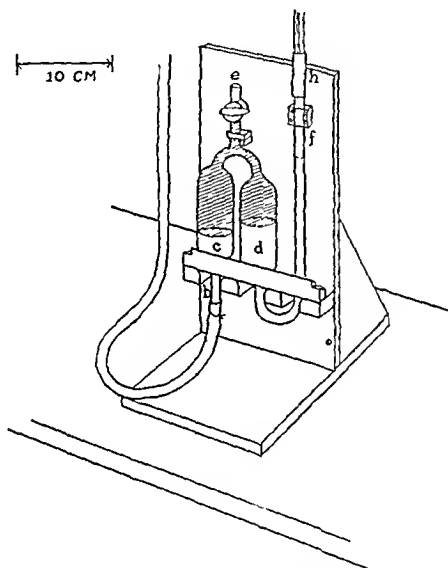


Fig 2—The mercury trap

The stopcock, M , connecting the compensating tube, B , with the outside air is of the type recommended by Carpenter.

R , and S are holders for the gas sampling bulb, Q . The leveling bulb is suspended from a hook behind the stand when not in use.

The leveling bulb, T , is raised and lowered by means of a cord and weight over a pulley. The pulley, U , is under the tension of a spring, s , to keep it from slipping. The weight, W , counterbalances to a great extent the weight of T . For aid in setting the levels the micro adjustment, V , is used. The bar, X , upon which the pulley U , is fastened, is swung up and down about the fulcrum, t , by means of the thumb screw, w , acting on the projection, y .

The fineness of the adjustment depends on the thread of the screw, w , the distance from U to t , and the distance from t to y . With the distances, as shown in Fig 1, a screw with sixteen or more threads to the inch is suitable.

A very annoying occurrence in gas analysis is the fouling of the mercury by the rubber tubing and the consequent contamination of the burette. To obviate this, some means must be used to prevent the mercury that comes in contact with the rubber tubing from entering the burette. For this purpose the apparatus shown in Fig 2 was designed and has worked excellently. The apparatus is mounted on a suitable stand. The bulb, d , is filled with mercury to the level, f , and a temporary petcock at h is closed. Water is then drawn into c through e by means of a leveling bulb connected at b containing mercury. By opening the petcock at h , and with proper manipulation of the leveling bulb and the stopcock, e , all air is forced from the apparatus. The tubing connecting the leveling bulb to b should be the same tubing that will be used to connect the reservoir, T , (Fig 1) with d (Fig 2). After the apparatus in Fig 2 is filled and connected to the burette A in Fig 1, the end of the tubing is passed up through the table and connected to T . It is best to bind stopcock e with tape to prevent the possibility of the stopcock from working loose. If the end, e , of the burette, A , in Fig 1, and the end of the glass tubing, h , of the apparatus in Fig 2 are made to fit snugly, very little mercury will come in contact with the rubber tubing. The bulbs, d , and c (Fig 2), should each have a capacity greater than that of the burette. For use with the burette described here a capacity of 75 cc is ample.

The author wishes to express his gratitude to Raymond Maas, departmental technician, for his kind assistance.

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NOTES ON CONSTITUTIONAL REACTIONS IN HAY FEVER THERAPY*

By HARRY S. BERTON, M.D., WASHINGTON, D. C.

THE preventive treatment of hay fever by desensitization with appropriate pollen extracts is at present enjoying a well deserved popularity. Despite certain limitations, this therapeutic procedure has a firm scientific basis. It is, therefore, natural that the profession at large turns readily and hopefully to the use of pollen extracts.

The schedule of the course of treatments with gradually increasing dosage first attracts attention. The administration of a large terminal dose is well come as the goal of achievement. Not enough emphasis, however, has been placed on the possibility of constitutional reactions resulting from the injection of pollen toxin to which the patient has been found sensitive. Constitutional reactions are distressing, and every effort needs to be exerted to minimize their occurrence. The physical discomfort of preventive treatments should not be permitted to suggest the discomfort of the disease itself.

There are two principal causes of constitutional reactions. The first and most common cause is an overdose of pollen extract. Individual sensitiveness shows a great variation. Every hay fever subject is a law unto himself. Only the subcutaneous injection of pollen extract furnishes the necessary information. It does not follow that the patient who exhibits marked cutaneous sensitiveness to pollen possesses a similar high degree of sensitiveness to small doses of pollen extract when administered subcutaneously, and vice versa. A local reaction with induration extending from four to five inches around the site of injection serves as a warning. Under such circumstances, it is advisable to repeat the dose until the tissues become less sensitive. I have had a few patients in whom the initial dose of five pollen units had to be repeated five times before any increase in dosage could be safely administered.

The second cause of constitutional reactions, though less common but of a more severe type than the first, is the accidental intravenous injection of pollen extract. Some authors advise that before the injection is made the piston of the syringe be withdrawn to determine whether or not a blood vessel has been entered. This procedure is not practical. Even though the withdrawal of the piston reveals the absence of blood, there is no assurance that in the subsequent manipulation, the needle point may not enter an adjacent capillary. The technique is entirely different in intravenous therapy. In the latter, the attempt is made to enter the lumen of a visible or palpable vein. In subcutaneous injection, the danger is always present of entering a capillary which is neither visible nor palpable.

The technique which I follow is to hold the skin of the upper arm tense with my left hand and make a sharp plunge with the needle of the syringe,

*Read at the Fifth Annual Meeting of the American Association for the Study of Allergy, Washington, D. C., May 16, 1927.

which is held in my right hand, for a distance of half an inch. The needle is then slowly withdrawn for a fraction of an inch. The object of this maneuver is to withdraw the needle point from the lumen of a vessel if by any chance one has been entered. Criticism may be made that on first plunging the needle point into the subcutaneous tissue a small vein or capillary may be transfixed. In that event, the withdrawal of the needle point may lodge the eye of the needle in the lumen itself. To eliminate this possibility a change in axis may be effected by a slight sideward motion of the needle. I am of the opinion that this procedure will appreciably minimize the hazard of accidental intravenous injection. It is also advisable to apply slight pressure after the needle has been withdrawn to observe whether any blood issues from the punctured wound. Constitutional reactions which follow intravenous injections are almost immediate in their effect and demand rigorous treatment with adrenalin.

In the treatment of hay fever subjects with pollen extract, two precautions must be observed: first to have on hand a solution of adrenalin, and secondly, to have the patient wait from fifteen to twenty minutes after the injection before dismissal.

A MODIFICATION OF THE BROWN APPARATUS FOR THE COLORIMETRIC DETERMINATION OF P_{H^+} †

BY WILLIAM H. WRIGHT, PH.D., AND H. G. HARDING, M.S. MADISON, WIS.

FOR the colorimetric determination of the hydrogen-ion concentration, the drop method of Haas¹ and Felton,² improved by Brown,³ has come into general use. The accuracy of the method and its advantages for use with small amounts of well-buffered and often turbid solutions are well known.

It is important for laboratory workers and especially for beginning students to visualize the P_H ranges of the indicators in common use. The arrangement of the indicators along a straight line scale does not show their ranges in relation to each other or permit a convenient arrangement of the glass cells for checking purposes. Where the indicator ranges overlap, the relations are shown much better if the scales are arranged parallel according to the working ranges of the indicators.

With such an idea in mind a set, which has proved very satisfactory, has been constructed. This apparatus is designed for routine laboratory use and is just as convenient as the more compact sets so generally used.

The appearance of the set without the reagents is shown in Fig. 1. The set, complete with reagents and having the buffer solutions set up for the ranges of brom cresol purple and methyl red, is shown in Fig. 2.

*From the Department of Agricultural Bacteriology, University of Wisconsin.

†Published with the Approval of the Director of the Agricultural Experiment Station, Madison, Wisconsin.

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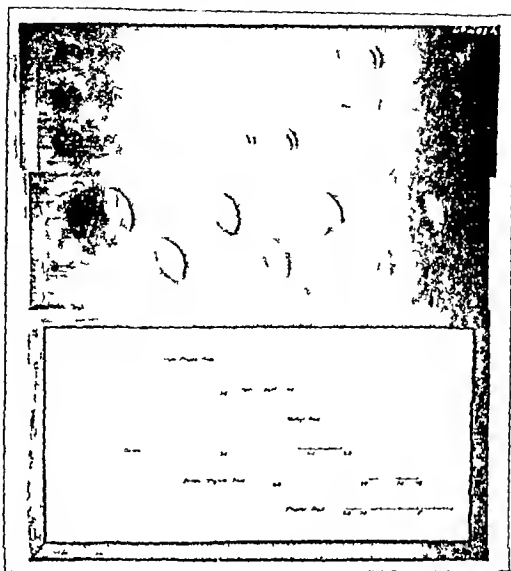


Fig 1—Appearance of the set with indicator showing the indicator ranges as they overlap on the P. H.

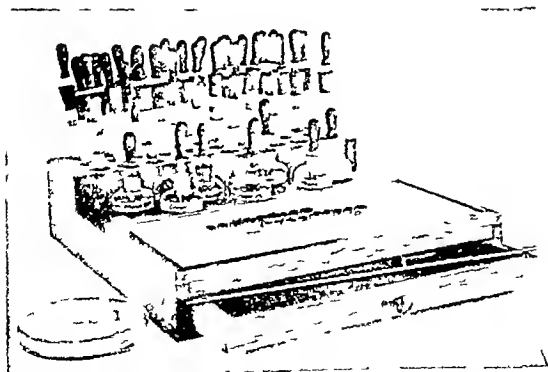


Fig 2—The complete set showing reagents in place. The overlapping arrangement of the indicator scales is shown in the two rows of cups.

The stained and varnished wooden base of the set is easily made in any carpenter shop. The drawer is convenient for storing the extra pipettes, the dilution loop, and a Petri dish containing the glass cups.

The indicator scales are ruled with India ink on a piece of white Bristol board which is mounted between two pieces of glass, the upper one being thin clear plate glass. The glass may be easily set in "Plasticene." Such an arrangement thoroughly protects the ruled scales and permits the wiping off of spilled solutions and the disinfection of the exposed surface.

A large number of tubes for the standard buffer solutions, etched at the top for marking, are plugged with cotton and sterilized. Clark and Lubs buffer solutions⁴ are prepared and after checking with the potentiometer, are placed in the sterile tubes. These are then heated once at 100° C for thirty minutes, sealed with paraffined sterile cork stoppers to prevent evaporation, and stored until needed. Rechecks with the potentiometer have shown a slight change in the P_H value of a buffer solution, due to heating, which does not make a noticeable difference colorimetrically when the solutions are compared before and after heating.

Distribution of the stock buffer solutions among many tubes and subsequent heating serves to protect them against mold growth. Such solutions may readily replace the ones in the set as they are used up or become inaccurate due to contamination or other cause.

The alcoholic indicators as prepared by Brown³ and modified by Taylor⁵ are used.

SUMMARY

An inexpensive apparatus, with an improved indicator scale, is described for the determination colorimetrically of P_H by the drop method. For laboratory and classroom work the apparatus is very satisfactory. The scale arrangement is very useful when checking determinations and visualizing the P_H range of indicators.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

CARCINOMA Contribution to the Problem of the Connection Between Blood Picture and Prognosis in X-ray Uterine Cancer. Hall E. Arch. f. Gynak., Feb. 3, 1926, cxviii, 708

The problem as to whether or not conclusions concerning the prognosis, in uterine carcinoma and in carcinoma in general, may be drawn from changes in the blood picture at the beginning of an x-ray treatment has gained greatly in significance since we began to regard the action of x-rays in carcinoma as conditioned by a general alteration in the tone of the organism, rather than as a purely local action of cell destruction.

Forty-three cases of cervical carcinoma, six of corpus carcinoma, and two of vulvar carcinomas, were studied two to five weeks after raying.

A favorable clinical course is evidenced at the beginning of an x-ray treatment by a relative, and a smaller, absolute increase among the lymphocytes, or else by an initial relative increase in the lymphocyte value which shows a brief decrease at the start of the x-ray treatment, and then quickly rises again past the normal value.

An initial low lymphocyte value and a further decrease or a small and slow increase at the start of x-ray treatment, is almost always indicative of a stagnation in the reparatory processes, and hence must be regarded as unfavorable for the prognosis.

The author does not believe that any especially definitive conclusions can be drawn from the behaviour of the eosinophile leucocytes, as he has seen in many primarily unfavorable cases, even when there was no helminthiasis or bronchial asthma present, high eosinophile values contrasted with lower values in markedly favorable cases.

BLOOD SEDIMENTATION The Diagnostic and Prognostic Significance of the Rate of Blood Sedimentation in Carcinoma. Guthmann H. and Schneider G. H. Arch. f. Gynak., Feb. 3, 1926, cxviii, 514

From a study of 300 cases of uterine carcinoma the authors conclude:

1. As an early diagnostic medium the blood sedimentation is of more value here than in pregnancy.
2. From the values of the blood sedimentation after two hours it is evident that an increase in the value is characteristic of a further extension of the carcinomatous process.
3. The material does not enable us to determine the degree to which a precancerous stage, in the sense of slight increase, may be made known.

Raying did not produce any characteristic changes in the sedimentation rate, the values showing a tendency to increase and later changing in correlation with the influence of the disease process.

Their general conclusions are:

Repeated and continued observation has shown that the blood sedimentation value parallels the clinical condition.

Improvement in the disease condition is accompanied by an approximation to, and deterioration by divergence from, the normal blood sedimentation value.

The first value observed before the treatment is not of itself of any prognostic value.

The intermediate values, observed during the use of the x-ray therapy, can provide an indication regarding the further course of the disease process.

Only values determined in a homogeneous blood status can be compared.

INTESTINAL INTOXICATION Plasma Chlorides in Acute Intestinal Intoxication of Children, Boyd, G L. *Am Jour Dis of Child*, April, 1926, *xxvi*, 514

The plasma chlorides were studied in sixty six cases of acute intestinal intoxication in infants. Hypochloremia, anticipated because of its association with other toxemias of similar nature, was found in less than one half of the cases studied.

No definite relationship between the chloride concentration and the toxemia present can be demonstrated. Extremely low chlorides were found only in severely toxic subjects, but not all those severely toxic had low chlorides, and even in fatal cases normal or high chlorides were found. It is quite obvious that the blood chlorides are of no prognostic importance.

No correlation was observed between the plasma chlorides and any other symptoms or signs of the disease. Fever had no effect whatever. Severe diarrhea was present in 84 per cent of those having low chlorides, but all severe diarrheas were not accompanied by hypochloremia. Accurate estimations of the amount of vomitus were not made, but in no subject with low chlorides did the vomiting appear sufficiently severe clinically to have been the cause of their reduction.

An interesting reciprocal relationship was noted between chlorides and proteins, and chlorides and sugar. Theories to account for these phenomena are purely speculative without further work. The chloride protein relationship would appear to be an expression of the disturbed acid base equilibrium which is present in this disease. It would seem as likely that the reciprocal relationship shown between chlorides and sugars would be due to the disturbed acid base relationship present. The coincident determination of the hydrogen ion concentration, plasma bicarbonate, proteins, sugars and chlorides will be necessary to clear up this problem.

BLOOD GROUPING The Inheritance and the Medicolegal Application of the Blood Group, Buchanan, J A. *Med Jour and Rec*, Mar, 17, 1926, *cxviii*, 354

Buchanan has always disagreed with the views of Ottenberg as to the possibility of determining paternity by blood grouping studies and the present paper is a review and summary of his position and the reasons for his contention that the blood group is practically valueless for determining parentage in a legal dispute.

HYPERGLYCEMIA Hyperglycemia Without Glycosuria in One Thousand Diabetic Patients, Stone, C T. *Jour Am Med Assn*, August 7, 1926, *lxxxvii*, 388

It appears justifiable from the material at hand to associate elevation of the renal threshold for glucose with hyalinization in Langerhans' islands in uncomplicated diabetes. When we have such a conception of the underlying cause of hyperglycemia without glycosuria, and regard hyaline changes as a late event in the course of the disease, such clinical findings sustained over a long period of time would appear to have a definitely unfavorable prognostic importance. This is in addition, of course, to the constantly increased likelihood of infections in those with hyperglycemia with or without glycosuria.

ACHYLIA GASTRICA The Etiology and Pathogenesis of Achylia Gastrica, Faber, K. *Am Jour Med Sc*, July, 1926, *clxxvii*, No 1, p 1

In this paper, illustrated with seventeen microphotographs, Faber reviews in detail the present knowledge of achylia gastrica and concludes that from the evidence at hand the condition has nothing particularly remarkable in its etiology and pathogenesis which marks it out from the diseases of other organs. It is due to disease of the gastric mucous membrane with its extensive glandular apparatus. When it is excited through nervous channels, it can only persist for a short time but apart from this it is due to the same causes which we know produce disease in other organs—direct irritation or hematogenous intoxication by bacterial toxins or autotoxins. With the exception, perhaps, of the initial stage of intoxication, the natural term for the disease is gastritis, by which is to be understood, not the old conception gastric catarrh, but a disease of the glandular parenchyma of an inflammatory nature.

The reason that the anatomic side of the question has come under consideration much later in this case than in that of other glandular organs like the kidneys and liver, is because postmortem changes take place so rapidly. As soon as one has learned how to avoid these the analogy is obvious and gastritis takes its natural place by the side of nephritis and hepatitis.

RENAL FUNCTION The Unitary Nature of Impairment of Renal Function Fishberg
A. M. Arch. Int. Med. August, 1926, xxviii, 239

The various forms of Bright's disease are usually divided, on a functional basis, into two great groups (1) In which sodium chloride is retained and clinically characterized by edema, and (2) in which nitrogenous bodies are retained and showing a tendency to uremia.

In this paper Fishberg presents evidence in support of the conception that no matter what its anatomic substratum, impairment of renal function is manifested by injury to all the excretory functions of the kidney and that when selective retention occurs in Bright's disease it is not due to inability of the kidney to excrete the retained substance but to intervention of an extrarenal factor.

He thus tabulates the blood chemistry findings in uremia:

Increased		Not Increased	
urea	phosphato	chloride	calcium
uric acid	sulphato	sodium	water
creatinine	urochromogen	magnesium	ammonium
indican	potassium (slightly)		

Fishberg concludes that:

Impairment of renal function involves all the excretory functions of the kidney.

No matter what the anatomic substratum—arteriosclerotic or inflammatory change, polycystic transformation or prostatic obstruction—impairment of renal function is always manifested in the same way, namely, by a lowering of the maximum concentration in which each of the individual urinary constituents can be excreted.

As impairment of renal function progresses, the maximum specific gravity attainable falls correspondingly. But no matter how severe the injury to renal function, the maximum specific gravity attainable does not fall below 1010.

When selective retention occurs in Bright's disease, it is not due to inability of the kidney to excrete the retained substance but to the intervention of an extrarenal factor.

The primary criterion as to whether or not a substance rises in concentration in the blood as a result of renal insufficiency is the normal value of the ratio average concentration in urine concentration in blood—for that substance. If this ratio is high (e.g. urea)

the substance will accumulate in the blood in renal insufficiency, while if the ratio is low (e.g. chloride) it will not.

Fully compensated renal hypofunction is revealed only by a lowered maximum concentration of the urine, for the detection of which a modification of Volhard's specific gravity test is used.

He performs the concentration test in the following manner:

The patient has his customary supper and then takes no fluids till four hours after waking the next morning. On waking he empties his bladder, this urine is discarded. He voids hourly for three hours and the specific gravity and volume of each specimen is recorded. Should he be unable to pass urine each hour one is omitted, so that there are only two specimens. It is best that the patient remain in bed during these hours. The highest specific gravity of the three specimens is considered as representing his maximum concentrating capacity. In health the specific gravity reaches from 1026 to 1034, usually about 1030. We have not seen true premia in

any case in which the maximum specific gravity reached 1.020 or over. A maximum specific gravity under 1.020 indicates impaired renal function. In the severest cases the patient is unable to concentrate above 1.010. Small amounts of albumin do not notably affect the result. In evaluating the results one must always be sure that edema or a serous effusion is not being evacuated, for this may simulate inability to concentrate.

The accompanying tables are given.

CONCENTRATION OF SUBSTANCES BY KIDNEY

SUBSTANCE	CONCENTRATION IN URINE	CONCENTRATION IN BLOOD,	NUMBER OF TIMES CONCENTRATED	CONCENTRATION IN BLOOD OF RENAL INSUFFICIENCY
	MG PER CENT	MG PER CENT		
Uric acid	60	2	30	Increased
Urea	2,000	30	65	Increased
Creatinine	75	2	35	Increased
Indican	1	0.05	20	Increased
Phosphate	150	3	50	Increased
Sulphate	150	4	40	Increased
Potassium	150	20	7	Slightly increased
Chloride	500	350	1.5	Not increased
Sodium	350	300	1	Not increased
Calcium	15	10	1.5	Not increased
Magnesium	6	3	2	Not increased
Water	--	1	1	Not increased

RELATION OF COMPENSATED AND DECOMPENSATED RENAL HYPOFUNCTION AND PRERENAL DEVIATION

	CONCENTRATING POWER	DILUTING POWER	BLOOD UREA
Compensated renal hypofunction -----	Impaired	Normal	Normal
Decompensated renal hypofunction ----- (renal insufficiency)	Impaired	Normal or impaired	Elevated
Prerenal deviation -----	Normal	Impaired	Normal or elevated

LABORATORY TECHNIC

ACETONURIA Concerning the Occurrence and Course of Acetonuria During Pregnancy,

Kleesattel, H. Arch f. Gynak. Feb. 3, 1926, cxvii, 717

A study of 2,000 pregnancies. Under normal conditions spontaneous acetonuria is very uncommon.

Acetonuria occurs regularly during labor, due especially to restricted nutrition. Regulated diet demonstrates a connection between nutrition intake and acetone output.

The etiology of acetonuria in pregnancy is formulated by the author as follows. Its basis is a carbohydrate deficiency in the nutrition, and its cause a functional change in the liver cells upon which rests the interaction of the carbohydrate and fat in the intermediary metabolism. It is easy to conceive of the inner secretory processes as playing a part in this functional change. This is indicated by the course of events in the praemenstruum, which in many respects resembles a pregnancy, in the frequent suppression of an acetonuria here by the use of insulin. The assumption is further supported by cases of pathologic pregnancy in which renal injuries are present, where the absence of acetonuria seems to be explained chiefly by the disturbance in the renal elimination.

The author's conclusions follow.

1. In pregnancy the fat oxidation requires larger quantities of carbohydrate than in nonpregnancy.

2 This change in metabolism corresponds to a change in the function of the liver cells, and apparently originates through the inner secretory actions of the placenta and the corpus luteum. It cannot be applied to the diagnosis of pregnancy.

3 If the diet is unsuitable the functional change may cause injury to the pregnant patient. An abundant carbohydrate addition is necessary in pregnancy, and the appearance of acetone in the urine is an indication of this necessity. Pregnancy toxicoses show only acetonurins.

4 A relatively insufficient carbohydrate addition causes distinct deterioration in pregnant subjects. During pregnancy it is well, and during labor it is necessary to restrict the fat and albumin addition.

BLOOD PLATELETS Blood Platelet Counts in Infants and Young Children, McLean S and Caffey J P. *Am Jour Dis Child*, December, 1925, *xxv*, 810

A careful and extensive study in which after a preliminary trial of four methods, that described by Wood, Vogel and Kamulener, (*Lab Technic*, New York J T Dougherty, 1922) was chosen because of its simplicity.

Four hundred platelet counts were made in 352 different infants and young children. Platelet counts in the newborn, in infancy and early childhood in forty-one showed a wide variation, fluctuating normally between approximate limits of 216,000 and 568,000.

The average count in fifteen newborn infants was 278,000, in seventeen normal infants was 359,000, and in nine normal young children was 341,000.

Platelet counts in ten premature infants showed an average of 246,000.

In fourteen cases of severe anemia in children less than twenty-two months of age, the average platelet count was 266,000. In these cases, a severe anemia was not accompanied by an abnormal platelet count except in three instances when it was definitely diminished.

In four cases of splenectomy there was a slight increase in the number of platelets during a long period of observation following the operation.

In three cases of acute lymphatic leucemia, there was a marked reduction in the number of platelets.

Diseases of the Respiratory System—In ten cases of acute bronchitis, the platelet counts were normal with an average of 352,000.

In sixty-nine cases of pneumonia the average platelet count was 328,000. Three patients showed counts of less than 200,000 and four had counts of more than 600,000. In sixteen cases of acute suppurative pleurisy the average platelet count was 334,000.

In four patients with pneumothorax, three of whom had suppurative pleurisy, the average platelet count was 338,000. In six cases of tuberculosis the average platelet count was 316,000. Two patients with whooping cough had normal platelet counts.

In five cases of active infantile scurvy the platelet count varied between normal limits of 216,000 and 440,000.

In fifteen cases of meningitis the platelet counts were normal. In eight cases of other inflammatory diseases of the central nervous system the platelet counts were normal.

In forty-four cases with various types of acute infection the platelet count was normal, excepting one count of 120,000 in a patient with septicemia.

In five of seven cases of congenital syphilis the platelet counts were normal. The platelet count in seven children following tonsillectomy was not significantly altered.

In twenty-four cases of acute intestinal indigestion, acute intestinal intonation and acute catarrhal colitis, the platelet counts were normal.

Conclusions

1 The platelet counts in normal infants and children fluctuate between approximate limits of 200,000 to 550,000 with a general average of 349,000. The number of platelets in the newborn and in premature infants is fixed within narrower limits and has lower averages 278,000 and 246,000 respectively. The platelet count in early life approximates the normal count in adults.

2 A reduction in the number of platelets occurred in only three of fourteen patients with severe anemia.

- 3 Removal of the spleen caused a slight increase in the platelet count
- 4 The platelet count was definitely reduced in three cases of lymphatic leucemia
- 5 In 173 cases of acute infection in infancy and early childhood, there was no significant alteration in the number of platelets
- 6 In the pathologic conditions studied in this group of cases, estimation of the blood platelet was of no valuable diagnostic and except in cases of acute lymphatic leucemia

VAGINAL FLORA A Study of Doderlein's Vaginal Bacillus, Lash, A F, and Kaplan, B Jour Infect Dis, April, 1926, xxviii, 333

A cultural study of the vaginal secretions of ninety eight pregnant women

Results of Vaginal Cultures in Ninety eight Pregnant Women

Doderlein's bacillus	13 (in smears—41)
Staphylococcus albus	91
Staphylococcus aureus	37
Diphtheroids	52
Yeasts (oidium)	47
Streptococcus viridans	33
Bacillus coli	30
Micrococcus tetragenus	8
Pneumococcus	2

The term Doderlein's vaginal bacillus includes a large group of organisms which, though related, have some differentiating characteristics. This fact makes it difficult to classify them.

Among the mediums used the most favorable for cultivating these organisms was found to be 1 per cent lactose in neutral broth.

Lactobacillus vaginalis, a provisional name for the Doderlein strain B studied has never been previously described.

The exacting cultural requirements and the incidence of the organisms in the normal vaginas of newborns and adults are facts upon which the hypothesis is based that the Doderlein's vaginal bacillus has a function in inhibiting the growth of pathogenic organisms by direct action or by maintaining certain conditions in the vagina by its action on the vaginal mucous membrane.

The associated organisms are not so virulent as when they occur in the pharynx.

STREPTOCOCCUS INFECTIONS The Bactericidal Action of Pleural Exudates VII Studies in Streptococcus Infection and Immunity, Gay, F P, and Clark, A R Arch Path and Lab Med, June, 1926, 1, No 6, p 847

This article describes first the histologic changes that take place in the pleura of rabbits as the result of the injection of sterile aleuronat or gum arabic broth mixtures. These changes are earlier and more marked in the parietal pleura and diaphragm, through which drainage of the pleural cavity takes place, than in the visceral (lung) covering. The changes are similar with the two substances employed and, owing to the delicacy of the tissue involved, show remarkable differences from normal as an acute inflammation followed by a more chronic condition in the nature of granulation tissue. Infiltration with polymorphonuclear cells on the first day is replaced by an intensified thickening due to mononuclear cells by the third day. By vital staining with trypan blue a preponderating number of these mononuclear cells are shown to be true clasmatoocytes or tissue macrophages.

The authors have previously discussed the cytology of the pleural exudate which we now find to be a reflection of the tissue changes here emphasized. They had shown that there is a definite relation between the cell picture, and presence or absence of an increased resistance to infection with a stain of *Streptococcus pyogenes* that produces in minute doses a fatal pleurisy. The correlation indicated that the clasmatoocytes are responsible for protection. This correlation is further emphasized by the tissue changes we have now described.

An acute inflammatory pleura is invaded and destroyed by virulent streptococci injected into the pleural cavity, whereas the same organisms disappear rapidly from the pleural cavity of animals with granulating and thickened pleural walls and are found enveloped within clasmatoocytes in a relatively undisturbed tissue. Phagocytosis of streptococci both in the exudate and tissues is almost exclusively by mononuclear cells.

The pleural wall may be thickened still further by repeating the injection of the non-specific substances or by injecting living streptococci. The degree of protection would seem roughly commensurate with the depth of granulation tissue that has been produced. The authors are unable as yet to say whether specific immunity produced by the streptococcus differs from increased resistance produced by nonspecific and sterile irritating agents in possessing some specific factor. In other words, whether all grades of local streptococcus protection are dependent simply on the number of clasmatoocytes present.

Preliminary experiments show that when one pleural cavity is treated sufficiently, the other cavity, although showing no histologic change in the pleural wall at the time of infection is nevertheless protected. This protection is accompanied by mobilization of clasmatoocytes in the pleural wall in an unusual fashion. There is further indication that the wall of the treated cavity is coincidentally stripped of its cells.

LEPROSY Serologic Analysis of Lepers. Sera Schöbl O and Ramirez J. Philippine Jour Sc, March 1926 xxix 305

Ninety-two lepers were examined serologically with the view of deciding certain doubtful points in the serology of leprosy. The question of complement and natural hemolysin content in lepers' sera toward guinea pig, sheep goat, and rabbit red cells was investigated. The content of hemolytic complement and its keeping quality in the lepers' sera were studied, antsheep and antimonkey immune hemolysis having been used in these tests.

Slight individual differences were found to exist in lepers' sera and in normal human sera alike as to content of natural hemolysins and complement, but no distinct quantitative differences were found between the sera of lepers and those of normal persons. The amount of hemolytic complement in the lepers' sera was found to be the same as that in the sera of nonlepers and is subject to individual variations.

As to the keeping qualities of the natural hemolytic complement, it was found that, in proportion to the original titer, the complement decreased practically at the same rate in the sera of lepers as it did in the sera of normal individuals.

BLOOD SERUM IN DISEASE A Study by Means of Ultrafiltration of the Condition of Several Inorganic Constituents of Blood Serum in Disease. Pincus J B, Peterson, H. A. and Kramer, B. Jour Biol Chem, June, 1926 lxxviii, No 3 p 601

In tetany, both infantile and experimental, there is a marked decrease in the "free calcium" of the serum.

In chronic nephritis, uncomplicated by uremic convulsions, the free calcium is normal. In one patient with convulsions it was definitely reduced.

The phosphorus (inorganic) is invariably high in parathyroidopriva tetany and in severe chronic nephritis with or without uremic convulsions.

The total protein concentration of the serum in experimental and infantile tetany is about normal.

Ultrafiltration of the serum at a reaction of P_H 4.7 shows that at this reaction the calcium is entirely filtrable.

The failure of the calcium concentration of the serum in tetany parathyroidopriva to increase after irradiation with the mercury vapor quartz lamp as it does in patients with infantile tetany makes it highly probable that infantile tetany is not a form of parathyroid tetany and this assumption is strengthened by the absence of any increase of the inorganic phosphorus of the serum of children with tetany comparable to that found in the serum of dogs after parathyroidectomy.

The fact that the free calcium of the serum may remain constant in patients with chronic nephritis even when the total calcium is definitely reduced, serves to explain why such patients do not develop convulsions

CARCINOMA Serodiagnosis of Cancer by the Reactions of Botelho, Lavedan, J Bull Acad de Med, Paris, May 25, 1926, *rev*, 543

The author tested the reaction on 273 patients but 73 of these cases were disregarded because the diagnosis was doubtful or because of alterations in the reagent which might have falsified the results. There remained 200 cases considered. The reaction was positive in all patients with cancer, negative in 33 patients with no malignant tumor, negative in 39 patients with cancer and positive in 17 patients without malignant growths. This gave 72 per cent of correct results and 28 per cent of false results.

He concludes that this reaction is not specific for cancer. It is not a reaction due to cachexia because it was frequently positive in cases without cachexia. It appeared to be frequently negative in advanced cases of cancer. There was no relation between the intensity of the reaction and the extent of the tumor. The number of correct reactions varied in cancers in different parts of the body. It was uncertain in cancers of the skin and mouth but there was a high percentage of correct reactions in deep cancers such as those of the uterus, breast and digestive tract and for these cases it may be of real diagnostic value. Also there were quite a few positive reactions in patients without cancers.

CARCINOMA Reduction Phenomena in the Serodiagnosis of Cancer, Mondain, C, Douris, R, and Beck, J Ann Inst Pasteur, Paris, May, 1926, *xl*, 431

The decoloration of the methylene blue in the reaction of Thomas Binetti is due to bacterial action. The necessary bacteria are supplied by the neoplastic extract, which immediately after fractional sterilization only contains resistant spores, but these spores soon vegetate.

Contrary to the statements of the authors, the cancer serum does not have the power of reducing methylene blue. The rapidity of the decoloration of the methylene blue is never proportional to the amount of the cancer serum, but on the contrary depends on the amount of the neoplastic extract, in other words, on the number of bacteria.

The incorrectness of the author's hypothesis is thus demonstrated. If the authors have been able in certain cases to note any differences between normal serums and cancer serums, it must be admitted that in these cases the normal serums had a greater aptitude to oppose the reduction of the methylene blue by the bacteria.

Without desiring to judge the value of the reaction, it may be stated that the interpretation given is manifestly erroneous.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond Va

*Immunity in Syphilis**

THE investigations of the last decade or so inaugurated by an epoch making trinity of discoveries—the demonstration of the *Spirocheta pallida*, the evolution of the complement fixation test and the discovery of trepanemine and neotsiphenemine have led to tremendous advances in the study of syphilis and have greatly augmented the knowledge available concerning its manifold aspects.

In this volume the twelfth of a series of Medicine Monographs (which are comprehensive reviews that adequately discuss a disease certain aspects of a disease or subjects that allow a better comprehension of disease processes) Chesney collects reviews, and summarizes the present status of the knowledge of immunity in syphilis.

Those who have followed the accumulating literature of the newer studies of syphilis have, of course, noted the extensive contributions of Chesney and Kemp to this subject and can appreciate therefore the fitness of the author for the thesis.

While the present volume is small and compact it contains a wealth of information which, as far as I know has not before been so succinctly presented under one cover.

Syphilis is recognized as a natural infection in man only but fortunately can be experimentally produced in its early stages at least in both monkeys and rabbits from studies upon which most of the newer concepts are founded.

In the human being neither racial nor individual immunity exist although some variations in the degree of reactivity to the infection seem to exist.

The syphilitic human being however gradually acquires a relative resistance against luetic virus which is more pronounced during the later stage of the disease. The production of active immunity by means of vaccination has not been accomplished.

The mechanism concerned in the production of immunity in syphilis is not yet understood.

Various antibodies such as agglutinins, precipitins and spirocheticidal substances have been demonstrated in the serum of both man and animals although their exact relation to immunity are not clear as yet.

There is some evidence as to the biological relationship of syphilis to yaws though this is so far unsettled.

The activities of the cells are of paramount importance the antibodies in the blood being of minor importance, if any.

The altered reaction capacity of the tissues sometimes manifested by allergy sometimes by anergy may be functions of the defensive reaction. It is doubtful if spontaneous sterilizing immunity in syphilis ever occurs. Man seems to be incapable unaided of eliminating syphilitic infection although he can react against it and influence its extent the reaction however at best being incomplete.

In thus touching upon the 'high lights' of the thesis, but little justice is done to the vast amount of material upon which this small volume is based.

Immunity in Syphilis. By Alan M Chesney M.D. Johns Hopkins Medical School
Cloth Pp 85 Price \$2.50 Williams and Wilkins Co Baltimore

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

The book is one of incalculable interest to all concerned with the study of syphilis whether from the clinical or laboratory point of view and should be purchased for its utility as a frequent source of reference

The typography and proof reading are excellent and almost devoid of error

Minor criticisms are directed toward what, in one sense, is a virtue of the volume—its compactness. So succinctly is the subject summarized, yet withal, so thoroughly, that the style at times, threatens to become laconic and one is tempted to hope that at some future time the author will expand the discussion. It is regrettable that there is no discussion of the effect of pregnancy upon the reaction to syphilis.

In view of the wealth of material which is discussed and summarized in this little book, an index would enhance its utility as a source of reference.

As it stands the volume deserves a place upon the bookshelf of every physician.

*Histologic Technic for Normal Tissues**

THE aim of this book is to give, in a compact form, the chief methods employed in the microscopic examination of human and other mammalian organs.

Part I consists of a brief discussion of the structure and composition of the cell in its relation to histologic technic, and an outline of the methods employed in histology.

In Part II there is a clear and excellent discussion of the fundamental principles of histologic technic, a very excellent section of "type methods" being described in detail.

In Part III various accessory methods are described: dark ground illumination, histochemical tests, injection methods, and methods of vital staining all being very well covered.

In Part IV methods for special organs, tissues, and cell components are presented in scriptum alphabetically, thus facilitating ready reference.

This section is very well done as is also the final section, Part V, in which are given methods particularly applicable to morbid histology, including methods for the determination of microorganisms and other parasites.

The book is not only well planned but well written and is well deserving of a place on the reference shelves of every laboratory.

A Textbook of Bacteriology†

IN 1910 Hiss and Zinsser presented the first edition of a textbook on Bacteriology, in which not only the laws and technic of bacteriology as illustrated by their application to the study of pathogenic bacteria were presented but also the complicated reactions taking place between the bacteria and their products on the one hand, and the cells and fluids of the animal body on the other were discussed from the standpoint of both student and practitioner.

The excellence of this volume led to its general use as a source of reference and it has long been recognized as a standard text, passing through many printings and five editions.

Thus, the sixth edition, entirely rewritten and revised by Dr. Zinsser is dedicated to the memory of Dr. Hiss and contains a section on the pathogenic protozoa written by Dr. E. E. Tyzzer, Professor of Comparative Pathology in the Harvard Medical School.

This textbook, the outgrowth of the Hiss and Zinsser familiar to all, requires no introduction. As stated on the title page, it is not only a manual of bacteriologic technic as applied to the study of diseases, but "a treatise on the application of bacteriology and immunology to the etiology, diagnosis, specific therapy, and prevention of infectious diseases for students and practitioners of medicine and public health."

**Histologic Technic for Normal Tissues, Morbid Changes and the Identification of Parasites*. By H. M. Carelton. University Lecturer in Histology, Oxford, and F. Haynes Demonstrator of Histology. Cloth. 17 figures. Pp. 398. Price \$5.00. Oxford University Press.

†*A Textbook of Bacteriology*. By Hans Zinsser with a Section on Pathogenic Protozoa by E. E. Tyzzer. Cloth. Pp. 1053. 181 illustrations. 6th edition rewritten, revised and reset. D. Appleton & Co. New York.

It is, therefore, a manual of infectious diseases and as such an invaluable source of information which should be in the hands of all who are in any way interested in disease.

The revision has been very extensive and thorough as necessitated by the extensive studies in the bacteriology and immunology of infectious diseases since the first edition sixteen years ago.

All of these studies and advances are reflected in the revision of the text which abounds throughout in practical applications to the clinical study of disease.

The book is divided into the following sections:

- I General Biology of Bacteria and the Technique of Bacteriologic Study (168 pages)
- II Infection and Immunity (124 pages)
- III Pathogenic Microorganisms (445 pages)
- IV Diseases Caused by Filterable Virus, the Exanthemata, and Diseases of Uncertain Etiology (60 pages)
- V The Higher Bacteria, Molds and Fungi (32 pages)
- VI Bacteria in Air, Soil, Water, and Milk (15 pages)
- VII Parasitic Protozoa (107 pages)

This is an invaluable volume which none concerned with the study of disease can afford not to have at hand.

*Tuberculosis**

IN THIS, the second volume of Trudeau Foundation studies, the authors present the results of their studies and experience 'to serve the needs of teachers, students, laboratory investigators, and technicians in tuberculosis institutions.'

There are numerous texts concerned with tuberculosis. In few, however, will there be found so much practical and utilizable information, succinctly expressed, as is to be found within the pages of this book.

The description of the tubercle bacillus in Chapter II, though laconically expressed, is extremely clear-cut. The technical methods for its isolation and study, clearly detailed in Chapter III, are mainly those which have been thoroughly tried. This chapter will be read with avidity by laboratory workers as will also the section on Diagnostic Methods (Chapter XIV) and that upon Serum Diagnosis (Chapter XV).

It is to be hoped that future editions, in addition to the technique, will also present the authors' evaluation of the methods described, especially the relatively newer procedures, such as the sedimentation and various flocculation tests. This would be a welcome addition and one of value to those to whom this book is addressed.

An excellent idea of the scope and character of the work is to be gained from the headings of the remaining chapters: Infection in Experimental Tuberculosis, Histogenesis and Development of the Primary Tubercle in the Lung, Reinfection, Immunity, Pre-disposition to Tuberculous Infection and Disease, Natural Tuberculous Infection, the Pathologic Anatomy of Human Tuberculosis—the Lung, Other Organs, Epidemiology, Prophylaxis, Tuberculin, and Experimental Therapy.

All of these are considered in a most practical and usable way. One wishes, however, that the chapter on experimental therapy, while clearly pointing out the technical and experimental errors to avoid and thus greatly smoothing the path of the worker, had been broadened to include the results of the authors' experience in this field for the guidance of others.

This book deserves a place in the library of every physician and, especially, those who are interested or engaged in the study of tuberculosis.

To the laboratory worker, whether particularly engaged in the study of tuberculosis are truly invaluable.

The physical and typographic appearance of the book is excellent and the illustrations or only as it is encountered in the clinical laboratory, will find the book invaluable.

Tuberculosis, Bacteriology, Pathology and Laboratory Diagnosis. With Sections on Immunology, Epidemiology, Prophylaxis and Experimental Therapy. By E. R. Baldwin, S. A. Petroff and L. S. Gardner. Cloth. Pp. 34 + 83. Engravings 4. Colored plates. Price \$4.50. Lea and Febiger.

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Editor-in-Chief WARREN T VAUGHAN, M D

Richmond, Va

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EDITORIALS

Anaerobic Streptococci and Puerperal Fever

IN 1843 Oliver Wendell Holmes, braving the medical Podsnaps of his day, published his epoch-making paper on *Puerperal Fever*

Mr Podsnap, be it recalled, was that eminently respectable and highly self-satisfied individual in Dickens' *Our Mutual Friend* who "settled that whatever he put behind him he put out of existence" There was a dignified conclusiveness—not to add a grand convenience—in this way of getting rid of disagreeables which had done much toward establishing Mr Podsnap in his lofty place in Mr Podsnap's satisfaction

" 'I don't want to know about it, I don't choose to discuss it, I don't admit it! ' "

Braving, then, the Podsnappery of his generation, said Oliver Wendell Holmes "The disease known as puerperal fever is so far contagious as to be frequently carried from patient to patient by physicians and nurses "

On March 12, 1878, thirty-five years later, Pasteur first demonstrated in the blood of a woman sick with puerperal fever, the organism later to become familiar to physicians as the streptococcus and destined to be numbered among the most virulent of the bacterial causes of disease

Nineteen years later, in 1867, Lord Lister first enunciated the principles of antiseptics whereby the incidence of puerperal as well as other infections was eventually largely to be controlled and prevented, and with the development of bacteriologic technique the detection and study of the bacterial causes of disease changed the whole theory and practice of medicine.

There has always been discussion as to whether or not puerperal infection was a condition due to a pathologically specific organism, as is the case with diphtheria and typhoid fever, or an infection the bacterial etiology of which was governed by the flora of the genital tract normally present and introduced in one way or another into the uterus and thence into the circulating blood.

Undoubtedly there are many cases in which the purely saprophytic organisms of the vagina have etiologic roles fortuitously thrust upon them, just as there are many more cases in which streptococci are consistently found. There are still other cases in which, despite the clinical evidence of bacteremia, blood cultures are sterile, due, as has been suggested and will be discussed below, to the fact that anaerobic methods are not routinely used.

In a study of the bacterial flora of the vagina Kronig,¹ in 1895, demonstrated the presence of anaerobic streptococci, a finding fully corroborated by the more extensive investigations of Wegelius in 1908. At first regarded as saprophytes, though later shown to be true parasites, the importance of these organisms in the production of puerperal infections was first emphasized in 1910 by Schottmüller,² who described an anaerobic streptococcus to which he gave the name *Streptococcus putridus*, which he proved was not a terminal or postmortem invader, and which he believed to be of extreme importance as a frequent factor in the production of puerperal infection, especially following abortion.

In recent communications Schwarz and Dieckmann^{3, 4} comment upon the paucity of American medical literature upon this subject finding, up to 1926, only the report of Little,⁵ and but few references in the English literature.

It is their conviction that the importance of anaerobic streptococci in puerperal infection has been overlooked and that the statements in textbooks referring to its occurrence as "occasional," and referring to Schottmüller's claims as "too sweeping" are unintentionally misleading because of the fact that but seldom are the blood cultures taken in this condition incubated under anaerobic conditions.

These convictions are based upon investigations by these authors commencing in 1924 at which time they began the systematic study of anaerobic blood and uterine cultures.

In 165 uterine and blood cultures in suspected infected cases *Streptococcus putridus* was encountered 46 times and anaerobic streptococci, presenting slight cultural variations from Schottmüller's organism, were found 21 times, an incidence of 51 per cent.

In their second communication they report in toto their experience of twenty-six months, during which time in 200 blood cultures of 68 puerperal patients,—45 of whom had puerperal infection,—anaerobic streptococci were

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—R A K

Approved Clinical Laboratories

THE efforts of the American Medical Association in connection with the standardization of hospitals for approval have resulted, as is well recognized, in much that has been of value to the hospital pathologist and have greatly aided in the improvement of the equipment, personnel, and general atmosphere of the hospital laboratory.

Largely as a result of the realization by the profession at large, and especially by the clinical pathologists, of the necessity for a similar standardization of laboratories in general a movement has been initiated to ascertain for the benefit of the patient and the use of the physician which of the almost innumerable laboratories now purporting to make clinical laboratory examinations for physicians are of a type and calibre warranting their utilization and support.

As everyone knows within recent years there has been, and especially in certain parts of the country a veritable mushroom growth of laboratories, some of which are entirely commercial in their aspect others inadequately equipped and manned, and still others catering to the laity, cultist, quack, and physician alike.

It is certainly the desire of the physician to secure from the clinical pathologist the best that clinical pathology has to offer in the study of the patient, and it is the contention of the pathologist that more than laboratory equipment or technical ability is required in the making of a pathologist who is in the last analysis, a physician practicing a specialized branch of medicine.

Both the physician and the pathologist therefore, must be, and should be, interested in the list of approved laboratories recently published in the Journal of the American Medical Association.

It is to the interest, and should be regarded as the duty of every clinical pathologist

- 1 To scan the list carefully,
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found in 11 cases as compared with other pathogenic bacteria in 6 cases. Uterine cultures showed aerobic pathogens in 12 cases and anaerobic streptococci in 35 cases, more than one type of organism being isolated from either blood or uterus in comparatively few cases. In all these cases the organisms found in the blood were also found in associated lesions, such as endometritis, pelvic thrombophlebitis, etc. It thus appears that, if anaerobic cultures are employed, anaerobic streptococci will be encountered in puerperal infections with greater frequency than has hitherto been thought to be the case and that such cultural methods should be employed as a routine in the presence of this disease.

Schwarz and Dieckmann stress the practical importance of these findings in connection with the treatment of what is admittedly a disease of ominous prognosis and extremely high mortality.

In many of the instances of puerperal infection due to anaerobic streptococci the organism remains confined to the endometrium. Early in the disease, therefore, while the infection is rather superficial much can be done to limit its spread by digital (or blunt curette) removal of retained secundines or clots followed by potassium permanganate (1:4000) douches.

The later spread of the infection and its transformation into a bacteremia is a sequel of uterine and pelvic thrombophlebitis, and when anaerobic streptococcemia occurs the result is almost invariably fatal.

Because of this fact Schottmuller, at the suggestion of Bumm, proposed ligation of the ovarian and internal iliac veins when signs of progressive thrombophlebitis developed (repeated chills after emptying the uterus, tender bands in the parametrium).

His results with this procedure were not very gratifying, and the method was in disrepute until again reviewed by Miller⁷ in 1917, and again reported upon by Baldwin⁸ in 1922.

In addition to emphasizing the necessity,—for the further advancement of knowledge of puerperal infection,—of routine *anaerobic* blood and uterine cultures, Schwarz and Dieckmann comment upon the value of blood transfusion and forced nutrition as means of supporting the patient, and conclude with the following statement:

“We have come to the definite conclusion that we shall in the future cases of pelvic thrombophlebitis due to anaerobic organisms, particularly the *Streptococcus putridus*, attempt ligation of all pelvic veins. If the patient's condition justifies further procedure we shall remove the infected uterus with the tubes and ovaries, both to remove the infection of the uterus and to limit the degree of pelvic edema which results from ligation of this kind. * * * We feel now that if the best results are to be obtained from such a procedure, it must be done as soon as the organism has been recovered from the blood stream in connection with a chill.”

Whether such a plan comprises a satisfactory method of an admittedly virulent and fatal infection, only the systematic studies of the future will tell.

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4 To secure copies of the questionnaire upon which qualification is based either for his own use or to place where needed, and

5 Finally, to give to this long-needed movement all the support in his individual power

Particularly should this be done by the hospital pathologist who may emphasize the importance of this movement to the profession at large without incurring the suspicion of advertising or "drumming up business"

—R A K

material and a somewhat larger series of experiments might have shown the same effects, however, the point to be emphasized is that absorption by intracutaneous injection may be very rapid and considerable, at least equalling that of the intramuscular route. The effect on the blood pressure from intracutaneous injection may be very rapid for in those experiments in which it was measured in the first minute or two after the injection, the maximum rise occurred in this period, in 6 of 8 injections the rise was from 3 to 21 mm in two minutes, and in 12 of 14 injections the rise was from 4 to 40 mm in two minutes. Such a rapid rise may occur also from the intramuscular route but very seldom from the subcutaneous. Fig 2 illustrates the effect on blood pressure in a nine year old girl who reacted well to all methods of injection. Fig 3 is an example in which the blood pressure rose much more from the intracutaneous injection than from the intramuscular or subcutaneous route.

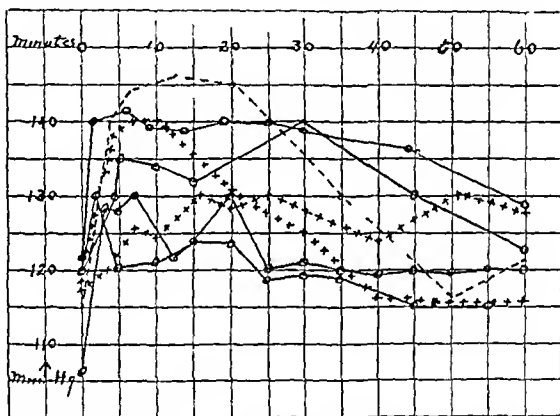


Fig — Blood pressure record in a 9 year old girl dose 0 mg epinephrin

o-o-o intracutaneous

- - - intramuscular

+ + + + subcutaneous

The reaction to subcutaneous injection was unusually large

The subjective and objective symptoms were in most patients roughly proportional to the vascular effect i.e. the more pronounced the higher the blood pressure. However there were exceptions, either a considerable rise in pressure without subjective symptoms or vice versa. The subjective symptoms and the tremors usually terminated before the blood pressure returned to normal often they ceased a considerable time before this, but occasionally persisted from thirty five to forty minutes. As the patients were young children the subjective symptoms were not always clearly expressed. When present they consisted of slight dizziness or headache or such phrases as "a pounding inside" (cardiac palpitation), "the bed wiggles" (tremor) or "I don't know what makes me so tired". The objective signs which never amounted to distress were tachycardia and usually a distinct

tissues of the same region. All subjects, except a few infants who received 0.1 mg, received the standard dose of 0.2 mg (0.2 cc of 1:1000 solution). Twenty-four hours at least elapsed between experiments. As a comparison of results of the different methods of administration of epinephrin is of real value only in the same subject (not in one subject as compared with another), this uniform dose suited the purpose as well as a dose by weight. The blood pressure was taken by the usual cuff method and recorded frequently during the first five minutes and then every five minutes until it returned to the normal. Control injections of 0.2 cc of 0.25 per cent trisecol solution in normal saline were made in each subject to learn whether fear of, or pain from, the injection had any influence on the blood pressure, and in but one case was any effect noted—a three-year-old negro boy's blood pressure rose from 112 to 134 mm from fear of the injection, and to 155 mm immediately after the injection but returned to normal within one minute. In subsequent injections of epinephrin no fear was manifested, and there was no immediate rise in blood pressure. It may be well to state that while an

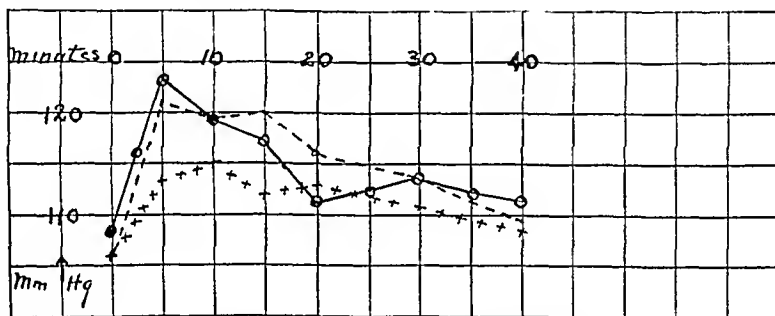


Fig 1—The average blood pressure records of all experiments
 —○—○— intracutaneous injection (22 experiments)
 - - - intramuscular injection (15 experiments)
 + + + + + subcutaneous injection (9 experiments)

occasional child protests against the first injection, there is seldom any objection to the subsequent ones, in fact it is not uncommon to have their associates ask to share their experience.

Effect on the Blood Pressure—The average maximum systolic blood pressure rise is charted in Fig 1. This maximum rise was usually reached within five minutes from the intracutaneous and intramuscular injection, occasionally somewhat later, and in about ten minutes by the subcutaneous route. The striking feature of the curve is that the average rise in pressure was somewhat greater by the intracutaneous than by the intramuscular or subcutaneous route. It is, of course, well known that epinephrin is fairly promptly absorbed from intramuscular injection and considerably more slowly from subcutaneous injection. The average maximum rise from the intracutaneous injections was 16 mm (extremes, 4 and 38 mm), by intramuscular injections, 15 mm (extremes, 7 and 28 mm), and by subcutaneous injection, 10 mm (extremes, 0 and 26 mm). The blood pressure was fairly well sustained above the normal, usually from forty to fifty minutes, occasionally somewhat longer. The differences between the intracutaneous and intramuscular routes are im-

subcutaneous injections also gave about as prompt and marked a reaction as the intracutaneous and intramuscular but of less duration while the other was less marked. This was the most marked reaction seen for the subcutaneous injections which as shown elsewhere were usually less than with the other methods of injection. The effect on the blood pressure is charted in Fig. 2.

DISCUSSION

The prompt action of epinephrin when injected intracutaneously is probably due to its rapid absorption. This may result from its being absorbed in relatively concentrated solution by the local vasoconstriction limits absorption, the latter factor having been suggested as the reason for the slight action from subcutaneous injection. Considerable care is exerted in making an intradermal injection and it is possible that because of this the solution gets into the venous or lymph channels of the skin readily. The pressure of the injection is considered to be a factor partly because a prompt rise in blood pressure follows the injection of epinephrin into the nasal submucosa under considerable pressure.

Vollmer² noted certain changes in metabolism in infants and children following the intracutaneous injection of 0.1 cc. of normal saline solution and attributed this to an influence acting through the nerve paths for the changes were the same when the circulation above the injection site was occluded. The epinephrin effects here reported were not due to a local action on the nerve mechanism for occlusion of the circulation by pressure on the arm prevented the action of intradermal epinephrin injection in the forearm. Such occlusion was maintained for periods of eight or nine minutes after the epinephrin injection in two subjects and the blood pressure was unaffected but release of the occlusion was followed promptly by a rise in blood pressure which did not attain the usual level, however.

It was not feasible in the present work to determine the rate of absorption by injection of dyes or other substances into the skin to learn the time of appearance in the urine for this would have necessitated cauterization nor would the presence of dyes in the vessels leading from the injection site have been evidence of absorption into the general circulation for while pale streaks due to constriction of the superficial vessels sometimes two or three inches long are often seen extending inward from the injection site such phenomena have been observed without any subsequent demonstrable effect of epinephrin on the general circulation.

Langstein and Vollmer³ and Levari⁴ have noted that intracutaneous injection of epinephrin causes a rise in blood pressure but give no data.

No therapeutic application is suggested for the facts herein presented. Possibly in urgent need epinephrin would be more serviceable when introduced intracutaneously than intramuscularly if the intravenous method could not be used. Because of the greater local discomfort associated with intracutaneous injections the method offers no advantages over the intramuscular route for general use.

pallor of the extremities and face which disappeared before the blood pressure became normal. The tremor of hand and fingers was often best noted by the observer placing his fingers lightly on the flexor tendons at the wrist of the subject. Pallor was seen in several older infants from the intracutaneous injection of 0.1 cc of epinephrin solution, in these the blood pressure was not taken. The blanching of the skin is a very striking phenomenon in infants in whom tremor, such as occurs in older children or in adults, has not been seen.

A summary of the results obtained in one subject as an example of a marked reaction by all methods of administration of epinephrin follows. A white female aged nine years, weight 51 pounds, convalescent chorea, no cardiac lesion, a well-nourished, quiet, nonexcitable, well-behaved child. Two

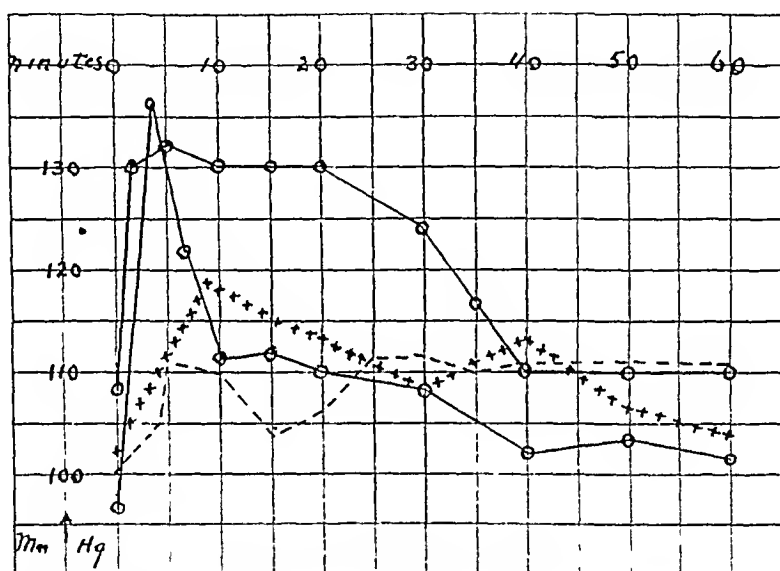


Fig 3—Blood pressure record in a 12-year-old boy dose 0.3 mg epinephrin

○—○—○ intracutaneous

- - - intramuscular

+++++ subcutaneous

The intramuscular reaction was less than the average in this instance

minutes after an intracutaneous injection of 0.2 mg of epinephrin the blood pressure rose from 120 to 140 mm and remained about this level during forty minutes, the heart rate increased from 78 to 104 and shortly to 120 per minute and remained at this rate for forty minutes. Within four minutes a pronounced, fine, rapid, uncontrollable tremor of the hands was present, accompanied by pallor of the skin, extremities and face, and to a less extent of the body, the child remarked that "there is a pounding inside and my head feels funny" but continued to be happy and smiling. These signs and symptoms persisted, with short periods of decreased severity, for about forty minutes. The effects were similar in a second experiment but somewhat less in two others. From a single intramuscular injection the effect was as prompt and marked as from the intracutaneous injection above quoted. One of two

METHODS

Only rabbits were used. They were kept on a diet of oats, cabbage, carrots, bread, and hay and were used at various times after feeding to eliminate the factor of digestion leucocytosis.

The usual method for counting blood cells was used. Only standardized instruments were used. Hare's solution was used for counting the erythrocytes, and 1 per cent acetic acid colored with gentian violet was used for enumerating the leucocytes. Cover glass preparations were treated with Wright's stain, and for the differential counts from 500 to 600 cells were counted. The cells were grouped as follows: neutrophils (pseudoeosinophils), lymphocytes, true eosinophils, basophils, monocytes, and myelocytes. For a detailed description of these cells reference may be made to the atlas of Kleiberg and Carl.¹²

Hemoglobin was determined by the Tallqvist scale. This is notoriously inaccurate but most closely approximated the color of rabbit hemoglobin. In view of the fact that accurate quantitative figures were not looked for, the desire being merely to determine whether or not the hemoglobin changed, I feel that this method answered the purpose. In no experiment was any marked or definite change found.

The red cells were counted in order to determine whether or not there was a change in blood concentration. In view of the fact that the greatest change obtained was a 17 per cent increase the average being about 8 per cent, and that the change in erythrocyte count did not always occur at the same time as the leucocyte count this procedure was not followed out in the majority of the experiments.

All drugs were injected subcutaneously.

Spleens were removed under aseptic conditions. It is realized that even after removal of the spleen much lymphoid tissue remains. However this procedure is necessary to determine the role played by the spleen in drug leucocyte change.

EXPERIMENTS

Lack of space prevents the reporting of the experiments in detail, only summary tables being included. The net maximum figures reported are the percentage changes above or below the absolute total leucocyte count change.

PROTOCOL I

SUMMARY OF ADRENALIN EXPERIMENTS WITH FED ANIMALS

EXPERIMENT NO.	NET MAXIMUM CHANGES						
	1	2	3	4	5	6	7
	SPLENECTOMIZED						
Neutrophile	+118.16%	+ 58.68%	+102.05%	+134.80%	+106.09%	+ 49.20%	+76.00%
Lymphocyte	- 80.34	+ 10.39	- 80.52	-139.17	- 36.43	- 44.20	-50.90
Eosinophile	- 19.26	-106.57	+ 63.23	+155.42	+120.71	+ 93.95	+34.77
Basophile	- 62.05	- 93.22	- 15.09	+122.67	+ 56.10	+193.39	-15.86
Monocyte	- 31.93	-112.80	+131.19	+ 26.42	- 23.93	- 33.08	-50.90
Myelocyte	- 78.97		- 56.32	+663.92			-90.90

PROTOCOL II

SUMMARY OF ESERINE ADRENALIN EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES	
	1	2
Neutrophile	- 24 35%	- 39 65%
Lymphocyte	+ 32 84	+ 44 25
Eosinophile	-130 99	+ 39 85
Basophile	-126 43	- 5 65
Monocyte	+ 41 13	-116 95

PROTOCOL III

SUMMARY OF PILOCARPINE EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES					
	1	2	3	4	5	6
					SPLENECTOMIZED	
Neutrophile	-54 27%	- 62 23%	- 48 77%	-131 36%	-87 53%	-132 78%
Lymphocyte	+12 64	+ 66 05	+ 56 90	+186 91	+57 98	+210 45
Eosinophile	-24 85	- 44 95	+ 54 32	-408 28	-99 99	- 0 33
Basophile	-50 28	+124 19	-159 32		-50 41	+857 52
Monocyte	+50 92	-229 02	+ 46 87	-182 25	+56 98	+ 10 27
Myelocyte			-113 87	-105 38		-283 78

PROTOCOL IV

SUMMARY OF ESERINE EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES	
	1	2
		1ST PHASE 2ND PHASE
Neutrophile	-192 14%	-42 00%
Lymphocyte	+218 15	+73 30
Eosinophile	+ 65 65	+20 60
Basophile	+ 52 50	-13 49
Monocyte	-219 35	-26 45

PROTOCOL V

SUMMARY OF ATROPINE EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES					
	1	2	3	4	5	6
				SPLENECTOMIZED		
Neutrophile	+12 98%	+107 93%	+ 21 22%	+ 64 04%	+27 78%	+105 35%
Lymphocyte	- 5 84	- 19 48	- 20 82	- 46 63	+14 37	- 87 94
				-137 99		
Eosinophile	-55 78	+ 7 55	+104 09	-133 63	+27 99	+ 38 66
Basophile	+90 65	- 48 20	+ 14 24	+ 28 74	+48 02	+ 12 63
Monocyte	-27 80	+ 00 05	- 15 05	-137 76	+45 95	-149 73

PROTOCOL VI

SUMMARY OF ACETYLCHOLINE EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES	
	1	2
Neutrophile	- 381 73%	-117 23%
Lymphocyte	+ 326 34	+158 67
Eosinophile	+ 427 66	+ 13 36
Basophile	+1275 72	- 28 84
Monocyte	- 326 34	-111 22

DISCUSSION

Two types of blood pictures may be recognized in the preceding tables (1) parasympathetic, as produced by such drugs as acetylcholine, tartrates and oxalates, arecoline, pilocarpine, guanidine, eserine and adrenalin after eserine, and (2) sympathetic, as effected by atropine calcium, and adrenalin. Atropine and calcium may be said to produce a negative parasympathetic picture, since they depress parasympathetic nerves.

Each picture presents certain primary characteristics and a number of secondary features which are less constant.

PROTOCOL VII

SUMMARY OF ARECOLINE EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES	
	1	2
Neutrophile	-16.93%	-55.41%
Lymphocyte	-46.62	+47.96
Eosinophile	+25.04	+80.65
Basophile	-6.93	+5.23
Monocyte	-56.26	-93.89

PROTOCOL VIII

SUMMARY OF GUANIDINE EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES			
	PHASE 1		PHASE 2	
	1	2	1	2
Neutrophile	-26.62%	+9.89%	+46.81%	+80.10%
Lymphocyte	+16.04	+27.28	-36.90	-30.69
Eosinophile	+59.89	+49.06	+34.87	-23.41
Basophile	-4.01	-3.33	+9.91	-11.39
Monocyte	+30.64	-14.30	-15.45	-19.19

PROTOCOL IX

SUMMARY OF CALCIUM EXPERIMENTS

EXPERIMENT NO	NET MAXIMUM CHANGES		
	1	2	3
Neutrophile	+43.80%	+20.2%	+20.59%
Lymphocyte	-19.88	-4.82	+41.71
Eosinophile	+37.00	+43.31	-48.34
Basophile	--	+0.68	-28.88
Monocyte	-35.00	-47.12	+63.13

PROTOCOL X

SUMMARY OF TARTRATE AND OXALATE EXPERIMENTS

EXPERIMENT NO	NET MINIMUM CHANGES		
	TARTRATE 1	OXALATE 2	OXALATE 3
Neutrophile	-50.81%	-32.27%	-2.74%
Lymphocyte	+155.79	+26.39	+23.00
Eosinophile	+52.38	+151.94	+473.04
Basophile	+3.93	+70.07	-9.58
Monocyte	-115.37	-68.26	+229

PARASYMPATHETIC PICTURE

The striking portion of this picture is a primary marked increase in the lymphocytes. On examining the individual protocols, it will be seen that there is a tendency to a secondary increase in the neutrophils and a corresponding decrease in the lymphocytes, i.e., a biphasic reaction. This, I am inclined to believe, is merely the result of fatigue of the nerves, and the result of an attempt to establish a normal balanced autonomic tone, in the attempt to do which there occurs excessive action just as in the healing of a wound there is excess production of tissue. It will also be seen from a study of the relative percentages that the increase in lymphocytes is, for the most part, at the expense of the neutrophils and vice versa.

With the exception of the tartrates and oxalates, which produce an activation of the parasympathetics by withdrawal of the calcium, all the other substances effect the change by directly influencing the peripheral parasympathetic apparatus, i.e., myoneural junctions or nerve endings as the case may be, either by sensitizing, as with eserine, or by stimulating, as with pilocarpine or adrenalin after eserine, and the other drugs.

TABLE I

THE CHANGE IN PARTITION OF LEUCOCYTES EFFECTED BY DRUGS INFLUENCING THE PARASYMPATHETIC NERVES

DRUGS USED	1	2	3	4	5		6		7
					PHASE		PHASE		
					A	B	A	B	
Neutrophile	-100%	-100 %	-100%	-100%	- 50% + 50%	+100%	-100%	+100%	-100%
Lymphocyte	+100	+100	+100	+100	+100	-100	+100	-100	+100
Eosinophile	+100	+100	+100	- 71 43 + 28 57	+100	+ 50 - 50	+100	-100	- 50 + 50
Basophilo	+ 50 - 50	+ 66 67 - 33 33	- 50 + 50	- 50 + 50	-100	+ 50 - 50	+ 50 - 50	-100	-100
Monocyte	-100	- 66 67 + 33 33	-100	+ 71 43 - 28 57	+ 50 - 50	-100	-100	+100	+ 50 - 50

1 = Acetylcholine

2 = Tartrates and oxalates

3 = Arecoline

4 = Pilocarpine

5 = Guandine

6 = Eserine

7 = Eserine + Adrenalin

+ = Increase

- = Decrease

Percentage represents portion of total experiments

TABLE II

THE CHANGE IN PARTITION OF LEUCOCYTES EFFECTED BY DRUGS INFLUENCING SYMPATHETIC NERVES OR DEPRESSING PARASYMPATHETIC NERVES

	ADRENALIN	ATROPINE	CALCIUM
Neutrophile	+100 %	+100 %	+100 %
Lymphocyte	- 85 71	- 85 71	-100
	+ 14 29	+ 14 29	
Eosinophile	+ 71 43	+ 57 14	+ 66 67
	- 28 57	- 42 86	- 33 33
Basophile	+ 57 14	+ 85 71	- 66 67
	- 42 86	- 14 29	+ 33 33
Monocyte	- 71 43	- 57 14	- 66 67
	+ 28 57	+ 42 86	+ 33 33

+ = Increase

- = Decrease

Percentage represents portion of total experiments

The lymphocytes are increased in 100 per cent of the experiments, while the neutrophils are decreased in 93 per cent and increased in 7 per cent of the cases

The change in the number of other cells is less constant. Eosinophiles are increased in 82.65 per cent of the experiments and decreased in 17.35 per cent. Monocytes are decreased in 70.75 per cent and increased in 24.25 per cent of the experiments, while the basophiles are increased in 69.05 per cent and decreased in 30.95 per cent of the experiments.

TABLE III

	INCREASE	DECREASE
Neutrophile	7.00%	93.00%
Lymphocyte	100.00	
Eosinophile	82.65	17.35
Basophile	69.05	30.95
Monocyte	24.25	75.75

SYMPATHETIC PICTURE

The sympathetic picture is somewhat different in that neutrophils are increased in 100 per cent of the experiments, while the lymphocytes decreased in 90.47 per cent. These findings are just the reverse of those in the parasympathetic picture and are characteristic. Secondly, there is an increase in the eosinophiles, basophiles and monocytes in about two thirds of the experiments. Table IV summarizes these findings.

TABLE IV

	INCREASE	DECREASE
Neutrophile	100.00%	0.00%
Lymphocyte	9.53	90.47
Eosinophile	65.05	34.92
Basophile	69.84	30.16
Monocyte	65.08	34.92

Analysis of Tables III and IV and the individual protocols shows that preponderance of lymphocytes and a corresponding decrease in the neutrophils is characteristic of the blood picture produced by parasympathetic drugs, while a preponderance of neutrophilic leucocytosis and a corresponding decrease in the lymphocytes is indicative of the action of the sympathetic drugs.

As can be seen from a review of the literature the fact that drugs are capable of disturbing the normal partition and distribution of leucocytes in the circulating blood has long been known and quite intensively investigated. Nevertheless there is some disagreement as to just what changes take place and what mechanism is involved.

Hess¹⁰ and Hatiegan⁸ believe that concentration of the blood affects the change in the white count with adrenalin. In my own experiments the change in red cells has never been greater than plus or minus 17 per cent and, further, the greatest change in the number of leucocytes does not always occur where the red cell change is greatest. It is quite difficult to see why concen-

tiation of blood following the use of one drug effects a lymphocytosis, while following another effects a neutrophilia. Early in this work, I dropped this factor as a consideration because of lack of any great change in the number of erythrocytes. It may be added that following the injection of pilocarpine the blood flows more rapidly than normal and that the clotting time is lengthened considerably. The opposite is true of atropine.

Harvey,⁷ Rous,¹⁷ Dixon,⁴ Dazzi,³ and Oehme¹⁶ consider the contraction of the spleen the cause of the increase of white cells in the circulation. Aschoff,¹ Billigheimer,² Grimm,⁶ and Kreuter¹³ state that contraction of the spleen is not sufficient to explain the changes. My own work seems to rule out the spleen conclusively, since virtually the same reaction was found after splenectomy as before, as can be seen from the tables. I conclude then that the spleen is of little, if any, importance in effecting drug leucocytosis.

Abel, quoted by Grimm, concluded that the hyperglycemia effected by adrenalin was the causative factor. While it is true that adrenalin produces a hyperglycemia (Sollmann, p. 442), some other mechanism must be considered in the production of pilocarpine leucocytosis, for McGuigan¹⁵ found that pilocarpine effected no change in glycogenolysis. Atropine also effects no change in sugar metabolism, although the differential count after this drug simulates that produced by adrenalin. However, a leucopenia is produced by atropine. Levine and Kalais¹⁴ determined the effect of insulin on the blood picture and found an increase in red and white cells, the latter being the greater. In view of the fact that pilocarpine effects a leucocytosis and atropine simulates adrenalin in the type of action without either drug influencing sugar metabolism, and the fact that insulin also increases the number of leucocytes while decreasing the amount of sugar, I conclude that a consideration of the factor is not imperative.

As has been mentioned before, some authors believed that the autonomic nervous system had something to do with the partition of leucocytes in the blood, but I do not believe any one has offered conclusive evidence. I feel that the blood may react in two ways to the injection of drugs. First, the production of a sympathetic picture characterized by an increase in the neutrophils, by a decrease in the lymphocytes, and by less constant increase in the other myeloid cells. This change may be effected either by increasing the activity of the sympathetic nervous system, as with adrenalin, or decreasing the parasympathetic nerves and leaving the tone of the orthosympathetics undisturbed as with atropine or calcium, second, the production of a parasympathetic picture characterized by an increase in the lymphocytes and a decrease in the neutrophils. The change in the other cells is less constant. We feel that the production of a parasympathetic, or in other words, a *negative* sympathetic picture, will account for this. I feel that increasing the parasympathetic activity does not necessarily decrease the sympathetic tone for all cells.

The parasympathetic action may be effected by pilocarpine, eserine, arecoline, tartrates, and oxalates, which amounts to withdrawal of calcium, and of adrenalin after eserine. In some previous work,⁹ I have shown that adrenalin after eserine is capable of acting on the parasympathetic nerves when

given in small doses. The fact that adrenalin after a sensitizing dose of eserine, that is, a dose which in itself will effect no demonstrable change, brings about a typical parasympathetic blood picture, makes me believe that this is conclusive evidence that the distribution and partition of leucocytes in the circulating peripheral blood are mediated through and are under the control of the autonomic nervous system. I further believe that the blood must be considered as a tissue and, as such, its function and activity must be under nervous control.

Whether this action is direct or mediated through the control of inorganic ions, such as calcium and potassium, I am not ready to draw absolute conclusion, but on the evidence of the calcium, tartrates, oxalates, and guanidine experiments, I believe such is the case. It is probable that this factor enters into the action of these nerves on all organs.

I offer the following reasons why I believe that the distribution of leucocytes in the peripheral blood is under the control of the autonomic nervous system.

1 The same blood picture is produced by such chemically different substances as pilocarpine, tartrates, eserine, nicotine, guanidine, and adrenalin after a sensitizing dose of eserine.

2 The same differential blood picture is effected by substances stimulating the sympathetic nervous system as adrenalin and by such substances as decrease the activity of the parasympathetic nerves as atropine and calcium.

3 The pilocarpine action on the blood is promptly and completely checked by atropine just as pilocarpine action on any other organ is stopped by this same drug, and further pilocarpine is ineffective after atropine has been given.

CONCLUSIONS

1 The distribution of leucocytes in the peripheral circulating blood of rabbits is controlled by the autonomic nervous system.

2 The partition of leucocytes in the peripheral circulating blood is an index of autonomic balance, the lymphocytes running parallel to parasympathetic tone and the neutrophils to sympathetic tone.

3 The increase of neutrophils takes place at the expense of the lymphocytes and the lymphocytes increase at the expense of the neutrophils.

4 With an increase in sympathetic tone there occurs a concomitant decrease in the lymphocytes.

5 With an increase in the parasympathetic tone there occurs a concomitant decrease in the neutrophils.

6 The spleen plays no part in the production of a drug leucocytosis.

7 It is possible and probable that this influence of the autonomic system is mediated through the medium of inorganic ions such as calcium.

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A CRITIQUE OF THE LIPASE "PICTURE" METHOD*

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FOR the past several years Falk and his collaborators have been studying the lipase activities of the extracts of various organs and tissues removed from recently killed animals. The method employed¹ is essentially as follows: the animal is killed and the organ is immediately removed and extracted with a definite quantity of water for a given period of time, portions of this extract are then incubated for a given time and at a given temperature with equi-molecular quantities of a number of esters, and the relative amounts of acid produced under these conditions are plotted graphically. This graph is then presented as a "picture" of the lipase activity of the particular structure which is being studied. Recently Falk² has stated his belief that these pictures, as compared with other methods of studying life phenomena, will perhaps "approach more closely from a chemical standpoint the properties and changes which occur in living organisms." After much disappointment with the method, I wish to record my reasons for believing that it perhaps has very little value at all.

1 *The Method Does Not Take Into Account the Serum Lipase*—Rona and Michaelis³ definitely showed, in 1911, that there is a serum lipase which differs in activity in the different animals. A little later Rona and Bien⁴ were able to show that this lipase is not the pancreatic lipase in circulation by proving that the two differ in optimal reaction and that they are affected differently by salts. Also the pancreatic lipase acts on both homogeneous and heterogeneous systems while the serum lipase is effective on only a homogeneous system; however, this does not justify us in not taking it into account here, for in those cases in which the esters are soluble in the extracts we are dealing with homogeneity. Nor can we assume it to be a constant quantity, for if we range ten splenic extracts side by side we shall see that no two are of exactly the same shade of pinkish-straw, however careful we may

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be in our procedure and in the washing away of surface blood. That is to say, the blood content per gram of spleen varies and therefore these extracts contain varying amounts of serum lipase.

This serum lipase is not taken into account at all in the method, but it is assumed that all of the lipase activity observed is due solely to that lipase which is indigenous to the organ under investigation.

2 *The Method Does Not Take Into Account the Profound Disturbances of the Whole Organism at the Moment of Death*—In unpublished experiments performed in this laboratory it was found that the subcutaneous injection of pilocarpine in the albino rat was apparently followed by an increased lipase activity of the spleen, as measured in terms of the activity of the spleen extract upon a number of esters. However when the normal lipase activity of the unstimulated organ was determined quantitatively in a large number of controls, it was found that these apparent pilocarpine increases were all well within the normal range which was astonishingly wide. The methods and findings in these control experiments will be briefly presented here.

EXPERIMENTAL METHOD

a Female albino rats of approximately the same age were used in groups of ten. Five animals of each group were killed by a crushing blow on the head, the other five were killed by the inhalation of concentrated ether fumes in a closed chamber.

b Immediately after killing the abdomen was opened and the spleen removed and weighed. Each individual spleen was then treated as follows: thoroughly pulped in a mortar without sand, washed into a flask with 100 cc of distilled water per gram of spleen, six drops of toluene added and the flask stoppered and placed in the refrigerator for twenty-two hours, removed from the refrigerator, filtered, 10 cc of the filtrate diluted to 200 cc with distilled water, the whole neutralized and 25 cc of this diluted and neutralized filtrate incubated in a stoppered flask with 5 millequivalents of the following six esters: benzyl acetate, methyl acetate, methyl butyrate, ethyl benzoate, ethyl acetate and benzyl benzoate. Toluene was added to each flask before placing it in the incubator.

c The period of incubation in the presence of these substrates was twenty-two hours at 39° C. The amount of acid produced under these conditions, titrating with $N/100$ NaOH with phenolphthalein as indicator, was taken as the measure of the lipase activity of a particular spleen after deducting for the necessary blanks.

This is a modification to meet experimental conditions of the method employed by Falk, Noyes and Sugiura.¹

TABLE I

Rat No	BLOW GROUP					ETHER GROUP				
	1	2	3	4	5	1	2	3	4	5
Rat weight in gm	19	202	188	178	21	106	110	119	197	212
Spleen weight in gm	0.48	0.47	0.45	0.72	0.4	1.46	0.56	0.58	0.48	0.6
Lipase activity in cc NaOH	2.15	1.10	1.70	1.45	0.9	1.35	3.10	5.50	2.75	0.40

PRESENTATION OF FINDINGS

The findings in one group of ten animals, five killed by a blow and five by ether, are shown in Figs 1 and 2 and in Table I. For purposes of simplicity only this one of many similar groups is presented here.

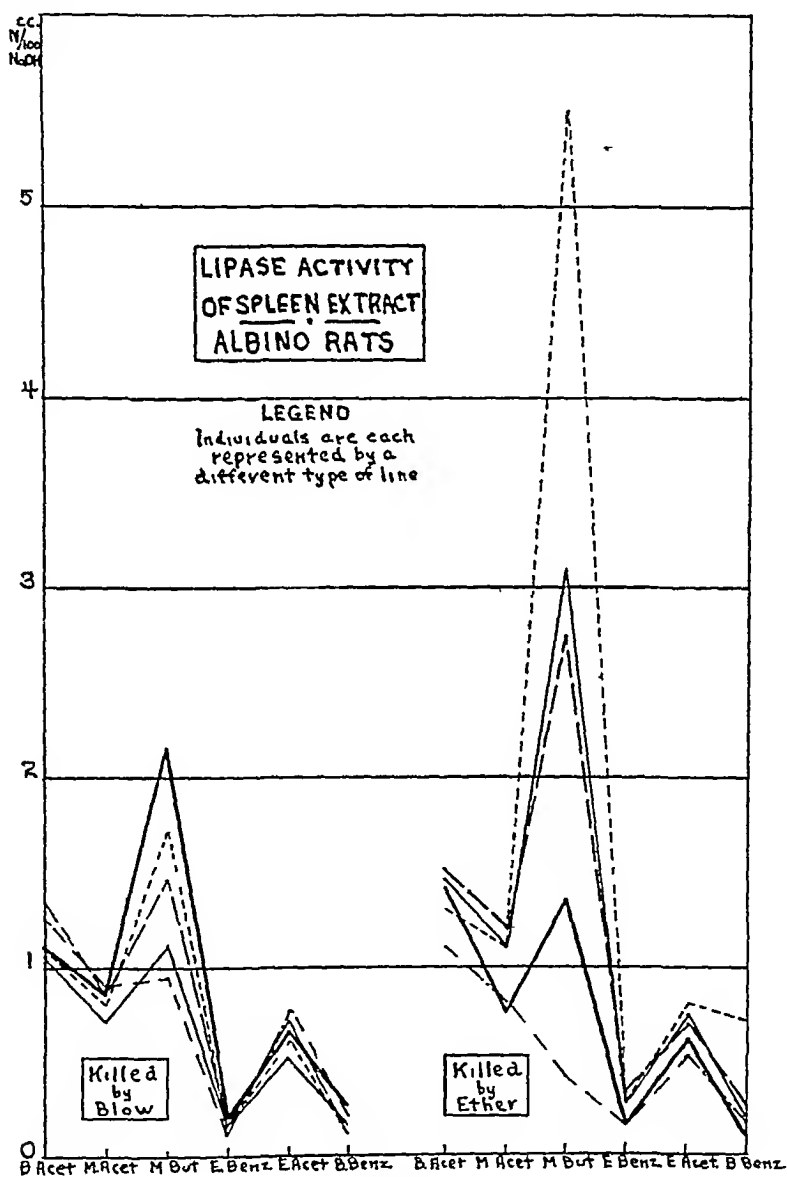


Fig 1

In Fig 1 the equivalent amounts of acid produced, in corrected cubic centimeters of NaOH as determined by titration, are shown as ordinates, the esters are placed as abscissae. It will be seen that there is a certain characteristic "picture" for all the animals in the group killed by a blow, but that very considerable quantitative differences exist between individuals of the

group. In the group killed by ether the same type of "picture" is again seen, but the quantitative differences between individuals are even greater than in the blow group.

In Table I the weight of each of the ten animals of the group is stated, together with the weight of its spleen and the lipase activity of that spleen in terms of cubic centimeters of acid produced by its action on methyl butyrate. That there is no constant relationship between the weight of the animal, the weight of its spleen, and the lipase activity of the spleen, is quite apparent.

In Fig. 2 the percentage variation from the average methyl butyrate activity of these spleen extracts is shown. The data graphically presented in this figure were obtained as follows: the five figures representing the ac-

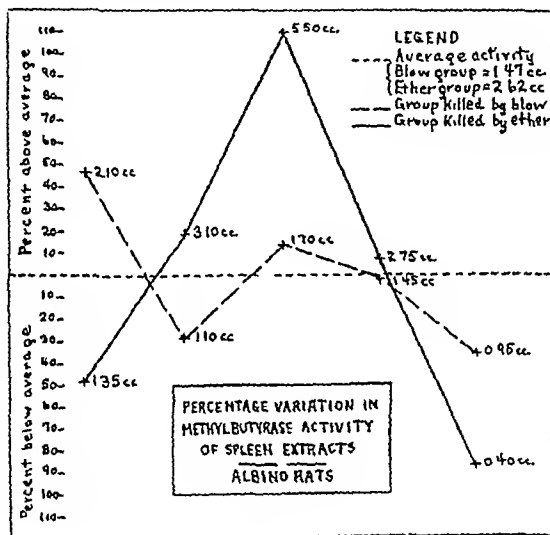


Fig.

tivity of the blow group on this particular substrate (as shown in Table I) were added, and this sum divided by five in order to obtain the average. This average figure (1.47 cc) was then represented by the dotted line extending horizontally through the middle of the figure, and the number of cubic centimeters representing the activity of the individuals of the group were placed in relation to this line according to scale. Percentages were then placed as ordinates above and below the line. The individuals of the ether group were plotted in the same way, the average figure here being 2.62 cc. It is shown in this figure that the five members of the blow group departed from the average of their methyl butyrate activities as follows: one, 46.2 per cent above, one, 27.2 per cent below, one, 15.6 per cent above, one,

136 per cent below, and one, 353 per cent below. In the ether group the individual variations were one, 484 per cent below, one, 183 per cent above, one, 1099 per cent above, one 49 per cent above and one, 847 per cent below.

Two ways of accounting for these great variations have occurred to me. They may represent the normal variation between the splenic lipase activity of individual animals, or they may be simply an index of the profound disturbance of all activities which takes place at death. If the former, then we

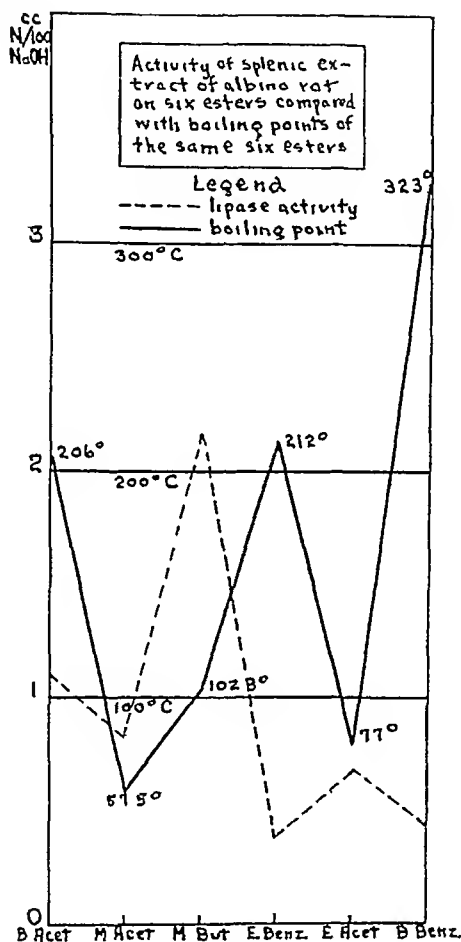


FIG 3

would seem to be entirely unvaried in accepting any set of determinations, whether they be presented in the form of the absolute or the relative quantitative figures, as representing even the most remote approximation to the typical picture of an activity, for in the statistical approach to a biologic problem the "typical" is the "average", and it would seem impossible to determine an acceptable average here. On the other hand, if these variations are merely an index of the profound functional disturbances at the moment of violently induced cessation of life, then we have a true "picture"

indeed, but it is only a picture of death. This latter would seem to me the more reasonable interpretation. That emotional disturbances cause considerable variations from the normal in many life processes is coming to be recognized more and more both in the laboratory and in clinical practice. Witness the scepticism of Barbour and Hamilton who have recently said, in discussing their new method of determining the specific gravity of fluids, "In view of the well established but neglected fact that relatively slight emotional disturbances are associated with anhydremia, one wonders how many really normal blood samples have ever been taken." Excitement increased the blood concentration in their dogs sometimes as much as 10 per cent. Must we not allow that the supreme emotional disturbance, which is death, may profoundly upset the enzyme activity of any organ or tissue?

3 *The Method Does Not Take Into Account the Escape of the Esters From Contact With the Extract*—The assumption is made that in placing definite quantities of an organ extract in contact with equimolecular quantities of a number of esters in separate tubes and maintaining the mixture at a given temperature for a given time, the resultant amounts of acid found will be directly comparable. That is to say, we assume that the enzyme will be in contact with the same number of molecules in all of the tubes. And so it would be if all of the esters were of the same boiling point. But they are not, as is shown in Fig. 3 where the curve of lipase activity of a particular spleen extract upon six esters is superimposed upon the curve of the boiling points of these esters. Obviously substances of such wide range in boiling point will vary considerably in their rate of evaporation. Exposing equal surfaces of the substances in calibrated tubes in the incubator for a given time will show the quite different rates at which they disappear. This being true the amount of activity of the extract upon the different esters is quite a different thing from the "picture" of this activity presented in the figure. Leaving out of consideration four of the esters which are not soluble under the conditions of these experiments it must be evident from observation of this figure that the comparative activity of the extract upon the two soluble esters, methyl acetate and methyl butyrate is not here shown for the reason that the methyl acetate is eluding by evaporation the destroying grasp of the lipase at a much faster rate than is the methyl butyrate.

This rate of escape of the various esters from the ester extract mixtures is a factor not taken into account in the method. Indeed if graphs, such as are presented by Falk and his collaborators and in this present paper are to be accepted as true pictures of the lipase activity of organs, then it would seem that a photograph of one's own hand with several fingers oscillating so rapidly as to appear only as blurs on the negative, might, with equal validity, be offered as a typical picture of the human presensile organ.

SUMMARY

The value of lipase 'pictures' obtained by the method of Falk et al is brought into question for the following reasons, which are developed in the paper.

- 1 The method does not take into account the serum lipase
- 2 The method does not take into account the profound disturbance of the whole organism at the moment of death
- 3 The method does not take into account the escape of the esters from contact with the extract

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HOUSEHOLD OBJECTS AS CAUSES OF HYPERSENSITIVENESS*

By SAMUEL M FEINBERG,† M D, CHICAGO, ILL

THE most important factor contributing to the successful management of the hypersensitive individual is the ability to correlate the results of the diagnostic skin tests with the objects in the patient's environment. Often this is a very simple procedure. But, just as often, the association between diagnostic test and clinical exposure is a much more difficult problem to determine. In many instances the diagnostic test has no apparent relation to anything in the patient's environment. This is due almost entirely to our ignorance of, and disregard for, the relation between raw material and finished commodity.

It becomes evident, therefore, that in the practice of allergy it is highly important to keep constantly in mind the various objects with which the patient is prone to come in close proximity, and to have some working knowledge of the substances that enter into the composition of these objects. It was with this thought in mind that an investigation was begun to determine the composition of some of the most important commodities and to determine the various objects in which any particular raw product was used. The facts presented herewith were obtained from several sources: standard textbooks on manufacture, textiles, etc., inquiry into commercial processes directly through the producer, United States Government Bureau Reports, and at times microscopic investigation. No attempt is made at this time to produce a complete outline of facts, but rather to present a few important illustrative reports with the idea that greater interest may be stimulated in the study of this important phase of allergy.

First of all, it is necessary to know in a general way what substances, capable of causing sensitization, are likely to enter into the make-up of some of our more important commodities, chiefly those of the home. Pillows are usually made of chicken, duck, or goose feathers, and sometimes of turkey or pigeon feathers. Cotton, kapok, silk floss, straw, or animal hairs are

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also used to stuff pillows. Mattresses are made of cotton, animal hairs kapok, straw, and cotton or wool felt. Most blankets are made of cotton or wool, or both. Domestic rugs are made of cotton or wool, oriental rugs may contain also goat hair, camel hair, linen, and silk. Some rugs are also made of vegetable fibers. Furniture may be upholstered with cotton, wool, silk or linen material, or with mohair (goat hair), the furniture stuffing is either feathers, cotton, silk floss, animal hair, wood shavings, or vegetable fibers. The chief materials used for clothing are cotton, sheep wool, linen, or silk. Of recent years camel hair and goat hair have been taking a prominent part in the manufacture of clothing. It is a notorious fact that furs are very rarely purchased under their original names. Most furs on the market are clipped, sheared, dyed and pulled in such a way as to resemble those which are more expensive. In cosmetics the ingredient giving the greatest amount of trouble as a sensitizing agent is orris root.

I will now proceed to enumerate more in detail the various raw materials and the manner in which they are used in the manufacture of finished articles. Many of the materials to be discussed have already been incriminated from time to time in the production of hypersensitiveness, others are still problematic, but are undoubtedly capable of producing hypersensitiveness, many of these undoubtedly do so, but we are unaccustomed to investigate them thoroughly.

ANIMAL HAIRS

The most important class of atopens is the group of animal epiderms, and of these the animal hairs play a substantial part. The animal hairs used for industrial purposes are sheep wool, various goat hairs, camel hair, cow hair, horse hair, rabbit hair, furs, and a few minor hairs, such as cat hair, hog hair, and deer hair.

Wool—The wool of the sheep is the most important animal hair universally used. It is employed in the manufacture of many articles of clothing both alone and mixed with other materials. It is also used in various ways in the more or less raw condition. Hypersensitiveness to wool has been reported in the literature. In my own experience I have, on several occasions obtained strongly positive skin reactions. Unspun wool is used for padding of robes and quilts, stuffing of mattresses, quilts, and sheepskin coats. Some of the better known materials for which wool is used are as follows:

Albatross	Wool gabardine	Mohair (imitation)
Astrakhan	Homespun	Tweed
Blankets	Jersey	Velour
Brandeloth	Mackinaw	Whipcord
Chunchilla (wool)	Melton	Padding for robes, quilts
Cravenette	Rugs	Wool for medical purposes
Doeskin	Sergo	Mattress stuffing
Felt (wool)	Suede cloth	
Flannel	Tapestry	

There are many other materials and purposes for which wool is used, but it is not necessary to enumerate them here. It is also important to remember that there are several varieties of sheep from which the wool is ob-

tained, and that specificity as to species may exist as regards sensitization. Archer divides sheep into thirty-two varieties. According to Matthews¹ sheep may be classified into three groups:

- 1 Ovis aries, domestic sheep, comprising the most important breeds
- 2 Ovis musmon, Mediterranean sheep
- 3 Ovis ammon, the Argali, from which Asiatic wool is obtained

Goat Hair —Goat hair has been reported as being responsible for some cases of asthma, vasomotor rhinitis, and dermatitis. The hair of the goat chiefly used in industry is mohair, which is obtained from the Angora goat. This animal, originally in Persia, is now being bred extensively in Cape Colony, Australia, and in the United States, chiefly in Oregon, California, and Texas.² The chief uses for mohair are^{1, 2}

Plushes for furniture upholstery, and railway cars. Here the term "mohair" is often erroneously used for other fabrics:

Coat linings

Men's summer suits

Dress goods

Braids and wigs

Rugs

Portières

Skins with hair used for carriage robes, muffs, coats, and trimmings for coats and capes

Cashmere is obtained from the Cashmere goat native to Tibet and the district of Kashmir in Northern India. This hair is used chiefly in the manufacture of Cashmere and Paisley shawls and Indian shawls. Most of the commercial fabrics designated by the term "cashmere" are various types of ordinary sheep wool.

Alpaca or *Lama pacos*, a species of llama, a domestic goat of Peru, yields a fine fleece of several colors which is used chiefly for natural colored alpaca yarns in the hosiery trade. The name "alpaca" is also given to a variety of wool substitutes. "Gorilla yarn" is a complex mixture of such hair fibers as alpaca, sheep wool, mohair, and cotton and silk wastes.

Camel Hair —This hair is now being popularized, especially in this country. It is usually employed in conjunction with other materials in the manufacture of fabrics. The Jager cloth is a camel hair fabric. Coats and sweaters are frequently made of camel hair. Blankets, rugs, dress goods, linings, and beltings frequently contain camel hair, brushes are often made of camel hair. I have observed instances of camel hair hypersensitiveness.

Cow Hair —Cattle hair is seldom used alone in fabrics on account of its short staple. It is found in coarse carpet yarns, blankets, and in a variety of cheap felted goods. It is used sometimes for the stuffing of mattresses and mixed in building materials, and enters into the manufacture of some animal toys. Positive skin reactions to cattle hair protein are rather common in my experience although it is not so easy to interpret its clinical significance in every case.

Horse Hair —This is used chiefly as stuffing materials for furniture and other upholstery. It is also used in mattresses and as padding for coats.

Rabbit Hair—This hair is very frequently employed in commerce although this fact is very seldom appreciated. Rabbit hair is the most common hair used in the manufacture of felt for hats. It is also found in other felts. In some parts of this country it is extensively employed as stuffing of pillows and even mattresses. Rabbit fur is also used in the lining of gloves, and on cuffs and collars. It is also of vast importance to realize that rabbit fur is very commonly altered by shrewing and dyeing and sold under names of better furs, such as sable, seal, chinchilla fox ermine, electric seal, and Hudson Bay seal.

Other Hairs—It is possible that some of the hairs of lesser importance may play an occasional role in causing hypersensitiveness. Badger hair is often used for shaving brushes. Mule hair is sometimes employed for the better variety of floor brushes. Various types of brushes are also made of hog hair.

FEATHERS

Chicken, duck, and goose feathers are the most common feathers used in large quantities, and constitute one of the greatest causes of allergic disease. These feathers find their greatest use in pillows and among foreign born peoples the use of feather beds is an additional source of trouble. Turkey, and occasionally, pigeon feathers are also used in pillows. Hats are often trimmed with clipped duck or goose feathers. Down is employed in the stuffing of furniture and sometimes in quilts. Ostrich feathers are used on hats and as a trimming for women's clothing. Marabou, the feathers of a species of African stork by the same name is used in the trimming of women's and infant's clothing. Egret or egrettes, feathers for headdress, obtained from various species of herons are a possible cause of hypersensitivity.

SILK

Silk, the fiber spun by certain species of caterpillars is of course, of animal origin. Hypersensitiveness to silk has been known to cause various manifestations of allergy. The silk fibers are twisted together to form threads which are used in weaving silk cloth. Broken loose, and tangled fibers the so called silk floss or waste silk are used for spinning purposes. It is important to realize that many articles and fabrics are sold under names suggestive of silk, but which contain only a small proportion of silk or none at all. The most important articles made of silk are waists, shirts, dresses, underwear, hosiery, thread, silk floss for pillows etc. rugs and upholstery. The following are some of the most common uses of silk.³

Broadcloth (silk)	Longee
Brocade	Rugs
Canton crepe	Silk poplin
Silk casement cloth	Radium silk
Chiffon	Wash satin
China silk	Tub silk
Crepe de chine	Tulle
Duchess satin	Thread
Foulard	Upholstery and tapestries

In this connection it is of interest to know that many cotton materials are sold under names suggestive of linen,⁵ such as

French linen
Killarney Linen
Linene
Near linen
Flaxon
Lanon

Other Vegetable Fibers—Jute, obtained chiefly from Bengal, India, is used in large quantities for gunny sacking, burlaps, matting, coat linings, cheapest clothing materials, paper materials, mixed with silk in making cheap satins velvets, and plushes

Kapok constitutes the seed hairs from nine varieties of trees belonging to the Bombaceae family. These trees are grown chiefly in the Dutch Indies and in Java. The term "kapok" is sometimes used as a misnomer of other vegetable fibers. Kapok is a soft, light, lustrous, silky material, but the fibers are very fragile and do not lend themselves to the making of textiles. It is used principally in pillows and mattresses, in life-saving belts, and in upholstery. Some recent attempts,¹ partially successful, have been made in spinning fibers from it. "Silk floss" or "floss silk" is a term sometimes used for kapok in bulk.

Bombax cotton is derived from a tropical cotton tree and is principally used for wadding and upholstery material.

There are many varieties of hemp, the most important being Manila hemp. Ropes and cables are the principal products of hemp. Sisal grass is used in large quantities for cordage.

Some of the less important vegetables fibers are ramie, used for fishing nets and gas mantles, aighan, or pineapple fiber, and coir, or coconut fiber. Vegetable down, the hair fibers of *Ochroma lagopus* of the West Indies, is used for stuffing mattresses and cushions. Pulu fiber and vegetable silk (*Asclepias* cotton) so-called milkweed or silkweed, are also used for bedding, pillows, and upholstery. Vegetable wool, obtained by special fermentative process from the green cones of the pine and fl, finds its chief use in mattresses, it is also mixed with cotton and wool in the making of some yarns. "Hygienic flannels," utilized at times for gouty patients, is made from vegetable wool mixed with sheep's wool. Various types of grass-like materials as palmetto, bloom-root, rush, palmetto leaves, wheat, rye, barley, and rice straw, and many others are made into brushes, brooms, hats, chair bottoms, floor mattings, sandals and stuffing for mattresses or furniture.

There are several groups of commodities which require somewhat special consideration due either to their importance as possible factors in sensitization or to the general ignorance that prevails concerning their composition. The chief of these are rugs, hats, and furs.

RUGS

Domestic rugs are made chiefly of cotton or wool or a combination of both. For cheap matting, various grass fibers are used. Oriental rugs⁶ are

made principally of wool and cotton but silk goat hair, camel hair, and linen are used frequently. Oriental rugs differ markedly in their composition, but in general each district produces rugs which are more or less similar in their design as well as in the materials that go into them. It is inadvisable in a presentation of this character to describe all rugs in detail, but the better known and more common types in each class will be considered.

The majority of Persian rugs are composed of cotton and wool, the warp and wool being usually of cotton and the pile of wool. Common examples in this class are the Golevan Serapi Kermanshaw Ispahan Saraband Musla bad and Kirman rugs. Among the better known rugs containing silk and linen in addition to cotton and wool are the Tabriz and Senna rugs. The Samls contain cotton linen and wool the Kandishans are made of pure wool or a combination of wool camel hair and goat hair.

The Turkish weavers practically never employ cotton in their rug making. Their carpets are made either of pure wool or combined with camel hair, goat hair, or silk. The Ghiordes and Mosuls are two prominent types which contain cotton occasionally. Some of the Ghiordes rugs contain silk. In the Mosul rugs goat hair is frequently and camel hair occasionally, found. The Ladi and Smyrna rugs are of pure wool. The Anatolians are of wool usually, but occasionally goat hair is used for the pile.

Caucasian rugs quite frequently contain goat hair or camel hair or both. Silk is not used among the Caucasian weavers. The Balu weaves contain cotton and wool and very frequently camel hair is used either for the warp or pile, goat hair is also used but less frequently. The Shirvan rugs constitute a well known class these contain goat hair at times. The Kazak rugs are made entirely of wool.

The Tuluoman rug makers are very fond of goat hair and use it extensively in their weaving. A good example of this are the Kliva rugs. Several of the Tuluoman districts use silk occasionally in their rugs, of these the most prominent are the Samarlands.

Chinese rugs are more or less similar in their composition, the warp and wool are made of either cotton or wool and the pile is either wool or silk.

HATS

Hats are commonly made of either straw fur or felt wool cotton, or silk. Straw hats are made of various grasses including the well known Panama. Fur caps are not much in vogue at present. But they usually masquerade under false names. From the standpoint of possible sensitization the felt hat is undoubtedly the most important. Hatter's felt is made usually from wool and cotton matted together with other animal hairs. Rabbit hair is used more for this purpose than any of the others. It is not at all improbable that some of the obscure cases of asthma or hypersensitive rhinitis, giving a positive skin test to rabbit hair owe their trouble to the constant source of exposure to the hair in their hats. Hare, cone, and nutria are also used. Fine felt hats are made from vicuna, camel's hair beaver and muskrat. silk is also used in felt hats.

ORRIS ROOT

Orris root is quite a frequent cause of hypersensitiveness, producing asthma, skin disturbances, and hyperesthetic rhinitis. The latter is a particularly frequent result in women. Orris root is the dried, powdered rhizome obtained from certain species of the Iris family—Iris Germanica and Iris Florentina.⁹ It is used for its scent, which is that of violets, and for its ability to blend with and hold other scents. The majority of face powders contain orris root, and it is this particular cosmetic that is responsible for the greatest mischief. In addition to face powders it is a common ingredient in perfumes and body powders, and is also found in tooth powder and sometimes in tooth pastes.

No attention is being paid to the possibility of other scents used in cosmetics as a possible cause of sensitization. According to Askinson¹⁰ there are more than 150 natural plant and animal odors used as ingredients in cosmetics. In addition there is a formidable list of synthetic products. The most common animal odors used are musk, from the muskdeer in the Himalayas, ambergis, from the pot-whale, castor, from the beaver, and civet, from the civet cat and muskrat.

MISCELLANEOUS

There are several groups of substances which will be taken up very briefly at this time.

Hypersensitiveness to glue has been reported in the literature on several occasions. There are two chief varieties of glue—animal glue and fish glue. Animal glue is made¹³ by boiling and steaming the hides of horses, cows, sheep, hogs and rabbits, and the bones and cartilages of sheep, calves, dogs, cats, goats, cattle, and horses. Leather wastes are also used. The common variety of fish glue is made from the entire fishes, isinglass is made from the air bladders of the fish. The chief uses for glue are for joining, as in carpentry work, cabinet making, etc., binding, as in papier mâché, in mineral colors in manufacturing of colored paper, bookbinding, sand papers, matches, sizing, cloth and paper, isinglass, gelatin and jellies, gelatin capsules, combs, and buttons. Gelatin is also used in the making of ice cream, candies, and commercial preparations for making home products. Gelatin is also used for clarifying liquors,¹⁴ and fish glues are also used for heavy solutions or pastes. Some mucilages and pastes are made from glue, others from gums which exude from the wounded bark of several plants—gum arabic, gum tragacanth, etc. Some pastes contain flour, starch, or dextrin.

Laundry starch, because of its finely divided state, may be a possible factor in causing allergic disease. In this country starch for laundry purposes is made from corn and wheat, in Europe potato starch is chiefly employed. The finest laundry starch is made from rice.

The fats and oils used in soaps are chiefly tallow and grease and coconut oil. In addition the following are also used—soya bean oil, cottonseed oil, red oil, palm oil, palm kernel oil, and peanut oil. Various perfumes are used to scent soaps.

Linseed oil is used extensively as a basis for paints. Soya bean oil and China wood oil are also used in large amounts. Turpentine which is a volatile liquid made by distilling the resin of the pine tree, is employed for thinning paints.

Pyrethrum hypersensitiveness is quite frequently encountered. It is used extensively in insect powders.

Hypersensitiveness to wood has been described on several occasions. This is practically always due to wood in a fine form, such as in sawdust or excelsior. Carpenters or individuals handling sawdust for cleaning or packing purposes may become sensitized to a particular variety of wood. Excelsior, which is used for packing purposes, stuffing of cheap furniture or mattresses is a possible source of allergy. Attempts have been made also to use excelsior for other purposes, such as for absorbing material, and for weaving into floor coverings.¹¹ The excelsior is made chiefly of aspen and yellow poplar pine, but, in various parts of the country a great variety of woods is used which are native to those districts.

Tobacco as a cause of hypersensitiveness has been observed in several instances. Tobacco is used chiefly for smoking purposes and for snuffing and chewing. Symptoms due to hypersensitiveness to tobacco may occur in non smokers as well as in smokers, the presence of tobacco smoke may be sufficient. Two examples of this sort have recently come under my notice.

FURS

The furbearing skins of animals are utilized extensively in this country, and there is hardly a home that has not some article of apparel made of fur. The costly furs are frequently sold under their correct names, but the ordinary varieties are sold under names suggestive of more costly furs. The only furs which the mass of people use are limited to a small group of skins which are practically unknown in the retail market as such.⁵ The cheaper furs are clipped, sheared, dyed and pulled so as to resemble those which are superior in wearing quality and warmth. The National Association of the Fur Industry in its 1925 Year Book⁷ describes the situation, outlines the remedies, and includes a dictionary of fur names which is quite extensive. For our purposes it is important to take cognizance of the fact that in considering furs as a possible cause of hypersensitiveness in an individual one must not accept the patient's word as to the kind of furs she possesses. Almost invariably it is something else.

An illustration of the foregoing may be cited. A man aged thirty, was seen by me in February, 1926, for an asthmatic condition of two years' duration. Protein skin tests showed repeated marked reaction to cat hair. Inquiry revealed that there were no cats at his home at any time. Further inquiry revealed the fact that the patient had a muskrat cap. He volunteered the information, however, that he noticed the asthma particularly in cold weather when he would go outdoors put on his fur cap and put the laps over his ears. He was requested to take the cap to a reliable furrier for examination with the result that the muskrat cap was discovered to be made of cat fur.

Attention is called to the fact that it is possible to identify the commercial fur hairs microscopically^s

The most important furs commonly altered and sold under names of superior kind are

SPECIES	ALTERED AND SOLD AS
Hare, dyed	Sable or fox
Hare, white	Fox
Rabbit, white	Ermine
Rabbit, white, dyed	Chinchilla
Rabbit, dyed	Sable
Rabbit, sheared and dyed	Seal, electric seal, Hudson Bay seal, muskrat
Muskrat, dyed	Mink, sable
Muskrat, pulled and dyed	Seal, Hudson Bay seal, electric seal, Red River seal
Mink, dyed	Sable
Marmot (woodchuck)	Mink, sable, skunk
Opossum	Beaver
Goat	Bear, leopard
Fitch, dyed	Sable
Kid, dyed	Lamb
Otter	Sable
Nutria	Seal, beaver, otter

SUMMARY

1 Attention is called to the importance of the relation between allergic disease and household objects

2 Data are presented showing the many, and generally unsuspected, finished products that are made from substances which are definitely known to cause hypersensitiveness

3 Attention is directed to the many unsuspected materials and variation in kinds and brands of well-known materials with which we may come in daily contact and which may possibly play a significant rôle in some cases of allergy

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PREPARATION OF NEUTRAL ACRIFLAVINE SOLUTIONS FOR INTRAVENOUS INJECTION†

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BURKE and Newton have discussed the factors that must be taken into consideration in the preparation of dye solutions for intravenous injection and in estimating the value of dye therapy in septicemia.¹ The present article describes experiments designed to determine the effect of some of these factors in the preparation of neutral acriflavine solution for intravenous injection.

EXPERIMENTS

Experiment 1—To determine the P_H of neutral acriflavine solutions when prepared with various solvents‡

A 1 per cent solution of neutral acriflavine was prepared in tap water, distilled water, 3 per cent sodium bicarbonate solution, Loewe's solution, buffered distilled water, and physiologic sodium chloride solution. The buffered distilled water was prepared by adding 0.300 gm. of potassium dihydrogen phosphate and 0.387 gm. of dipotassium hydrogen phosphate to 100 cc. of distilled water. The P_H of each solvent was determined by a Stwall comparator, the dye added, the solution filtered and the P_H again determined within an hour and after an interval of two days with a potentiometer§. The results are given in Table I. In all cases the addition of the neutral acriflavine caused the solution to become more acid. Most of the solutions became more alkaline upon standing.

Neutral acriflavine solutions for intravenous use are usually prepared in physiologic sodium chloride solution. The P_H of physiologic sodium chloride solutions varies with the manufacturer of the salt.² It also varies with the water used, tap or distilled water, age of the distilled water and with the application of heat.

It is evident that neutral acriflavine solutions as prepared and used in practice vary in P_H . Some patients may receive highly acid solutions and others receive solutions near the neutral point. The effect of a highly acid solution injected intravenously in an animal suffering with infectious acidosis may be serious. Also neutral acriflavine has less bacteriostatic action in an acid medium. Variations in acidity may account for some of the variations in results obtained by different clinicians. However, it probably cannot account for all the unfavorable results obtained in cases treated with acriflavine.

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‡Neutral acriflavine, Abbott, was used in all our experiments.

§The potentiometer determinations were made by Dr. J. R. Neller.

As a solvent for neutral acriflavine, in so far as the P_H is concerned, tap water is more desirable than distilled water. Proper buffering will insure a favorable reaction in the solvent and reduce the amount of change occurring as a result of the addition of the dye. A 3 per cent sodium bicarbonate solvent was found to have a P_H of 8.7 and also to be alkaline after the addition of the dye. The application of buffers in the preparation of solutions for intravenous use has been described by Mellon, Slagle, and Acree.³

TABLE I
RESULTS OF EXPERIMENT TO DETERMINE THE P_H OF NEUTRAL ACRIFLAVINE SOLUTIONS WHEN PREPARED WITH VARIOUS SOLVENTS

SOLVENT	P_H OF SOLVENT	P_H AFTER ADDITION OF DYE	P_H AFTER STANDING TWO DAYS
Tap water	7.6	7.05	7.73
Distilled water	6.8	6.22	6.29
3% sodium bicarbonate	8.7	8.06	8.59
Locke's solution	7.6	5.71	5.50
Buffered distilled water	6.8	6.37	6.39
Physiologic sodium chloride solution	7.3	6.04	5.34

Experiment 2—To determine the solubility of neutral acriflavine in various solvents

One per cent neutral acriflavine solutions were prepared in tap water, distilled water, buffered distilled water, physiologic sodium chloride solution, 3 per cent sodium bicarbonate, 4 per cent dextrose, buffered physiologic sodium chloride with 4 per cent dextrose, and Locke's solution. The solutions, with the exception of the tap water, were prepared with distilled water. After the dye was added the solution was warmed, rotated for thirty minutes, allowed to cool to room temperature, and then filtered through filter paper. The original container, filter paper, and funnel were dried and weighed. The residual matter on them was determined and from this was calculated the amount of dye going into solution. The results are given in Table II, and apparently indicate that the amount of dye going into solution varies with the solvent. This method does not determine accurately the amount of dye going into solution in each solvent. As the dye goes into solution some of the salts precipitate out. With Locke's solution the dried precipitate weighed more than 1 gm., i.e., more than the weight of the dye added. However, some dye remained in solution in the Locke's solvent as indicated by the color and antiseptic power. While this method does not, with the exception of distilled water, indicate the exact amount of dye going into solution it does indicate that a certain amount at least has gone into solution.

Experiment 3—To determine the stability of neutral acriflavine solutions when prepared with various solvents

One per cent neutral acriflavine solutions were prepared as in the preceding experiments. After the first few hours the dye solutions were placed in the dark and examined daily for thirty days. The degree of decomposition and precipitation was determined by comparing the staining power on filter paper and the amount of precipitation. A solution was considered

TABLE II

RESULTS OF EXPERIMENT TO DETERMINE THE AMOUNT OF NEUTRAL ACRIFLAVINE GOING INTO SOLUTION IN VARIOUS SOLVENTS

SOLVENT	ESTIMATED PERCENTAGE OF DYE IN SOLUTION
Tap water	0.796
Distilled water	0.9
Buffered distilled water	0.92
Physiologic sodium chloride	0.792
Buffered physiologic sodium chloride	0.794
3% sodium bicarbonate	0.488
4% dextrose	0.404
Buffered physiologic sodium chloride and 4% dextrose	0.68
Locke's solution	s

TABLE III

RESULTS OF EXPERIMENT TO DETERMINE THE STABILITY OF NEUTRAL ACRIFLAVINE SOLUTIONS WHEN PREPARED WITH VARIOUS SOLVENTS

SOLVENT	1	3	5	6	14	30 days
Distilled water	Unaltered				Unaltered	Slight precip
Tap water			Unaltered	Slight precip		
Buffered distilled water	Unaltered				Unaltered	Slight precip
Buffered physiologic sodium chloride	Unaltered			Unaltered	Slight precip	Heavy precip
Physiologic sodium chloride	Unaltered			Unaltered	Slight precip	Heavy precip
4% dextrose	Unaltered	Slight precip	Heavy precip			
3% sodium bicarbonate	Decomposed					
Buffered physiologic sodium chloride with 4% dextrose	Unaltered	Slight precip	Heavy precip			

unaltered if the stain on filter paper equalled that of a fresh dye solution and no precipitation could be observed when holding the flask up to the light.

The results are given in Table III. Of the solutions examined the sodium bicarbonate solution was the only one that began to break down within a few minutes after being prepared. When using this solution it is advisable not to allow it to cool and to inject as soon as the dye has gone into solution. All the other dye solutions apparently remained unaltered for hours and some of them remained so for days. It has been the practice to make up a neutral acriflavine solution in physiologic sodium chloride and store it away for future use. Whether such solutions are as satisfactory as fresh solutions is questionable. Our physiologic sodium chloride dye solutions became slightly cloudy, and a fine precipitate formed in from one to two weeks. In our opinion only freshly prepared dye solutions should be used until it has been shown that old solutions are just as satisfactory. The effect of age on the toxicity for body cells and on the bactericidal action is unknown to us. It is possible that dye solutions showing slight precipitation may still be serviceable, but this remains to be determined. Changes in P_H affect the therapeutic value of the dye and these may occur without visible evidence. When necessary to prepare a dye solution a considerable time in advance of its use it should be prepared in neutral distilled water and stored in that form. The

salts can be added just before the dye is to be injected. While the salts tend to cause the dye solutions to become unstable they can be used to advantage if properly applied.

Experiment 4—To determine the comparative bactericidal action of neutral acriflavine in various solvents.

A fresh 1:100 neutral acriflavine solution was prepared. Measured amounts were placed in flasks. Sufficient amounts of salts were added to make the required solvents. To each flask was then added additional amounts of solvent to make a dye dilution of 1:10,000. The solvents used were distilled water, physiologic sodium chloride, buffered distilled water, buffered physiologic sodium chloride, buffered physiologic sodium chloride with 4 per cent dextrose added, 4 per cent dextrose, 3 per cent sodium bicarbonate, and 3 per cent sodium bicarbonate in physiologic sodium chloride. To 5 cc of each of the dye solutions was added 0.1 cc of a twenty-four-hour culture of *Staphylococcus albus*. At intervals up to eighty minutes a loop of the dye organism suspension was plated out by the usual method, two plates being used. Not enough dye reached the second plate to inhibit growth. We expected to find some relation between the amount of dye in solution in the different solvents or the P_H and the bactericidal action of the dye solutions. However, the experiment failed to detect any material difference between the bactericidal action of the freshly prepared dye solutions. In all cases the organism died between the sixty and eighty-minute intervals. Whether comparable results would be obtained in the presence of blood or with older dye solutions remains to be determined. The use of greater dilutions of the dye might bring out differences in bactericidal action. It should be noted in this connection that in general more dye goes into an acid solvent and thus tends to counteract the reducing effect of the acid on the bactericidal action of the dye. Furthermore, the method used for determining the amount of dye going into solution does not in all cases indicate the exact amount of dye going into solution.

Various investigators have demonstrated an increase in the bactericidal action of acriflavine with an increase in alkalinity. Eggeith has shown that this increase is slight between P_H 6.2 and P_H 8.4. The bacteriostatic action, however, increases more rapidly with changes in P_H . The bacteriostatic action is the important factor in the blood stream.

The experiment was repeated with 1:100 and 1:1,000 dye solutions. In no case did the experiments indicate, in so far as the bactericidal action is concerned, a superiority of one solvent over that of the others. The experiment indicates the possibility of combining various substances with the dye without materially affecting the bactericidal action. The advisability of combining with the dye various substances that support the patient should be determined.

Experiment 5—To determine the comparative toxicity of neutral acriflavine in various solvents.

One per cent neutral acriflavine solutions were freshly prepared for each injection. The dye solutions were prepared in physiologic sodium chloride,

buffered distilled water, and 3 per cent sodium bicarbonate solvents. Injections were made in the marginal ear veins of normal rabbits. The dye solutions were injected at 37.5° C. over a period of ten minutes. Two or more rabbits were used for each injection as occasionally a rabbit will die immediately following a nonlethal dose of dye. The amounts of dye to be injected were calculated each day from the body weights. We used 5 doses rather than determine the minimum lethal dose for each solvent. The rabbits received from 25 mg to 35 mg per kilo. Preliminary experiments had indicated that rabbits would live following 4 injections of amounts of neutral acryflavine up to 20 mg per kilo in distilled water, physiologic sodium chloride, 3 per cent sodium bicarbonate and 3 per cent sodium bicarbonate in physiologic sodium chloride.

TABLE IV
RESULTS OF EXPERIMENT TO DETERMINE THE COMPARATIVE TOXICITY OF NEUTRAL ACRYFLAVINE IN VARIOUS SOLVENTS

RABBIT	SOLVENT	MG PER KILG PER INJECTION	DOSES	CHANGES IN WEIGHT				RESULTS
1	1 physiologic sodium chloride	25	1	+13	-180	-68	-290	Died
2	"	25	1	+7	-127	-68	-200	Died
3	"	25	1	+13	+13	-30	-35	Lived
4	"	25	5	+10	-20	-20	-60	Lived
5	"	30	3	+20	+15			Died
6	"	30	4	200	-360	-200		Died
7	"	30	5	-10	-5	+10	-20	Died, 3 days
8	"	30	1	-30	-10	-10	+10	Died
9	"	30	5	-60	-225	-215	-5	Died
10	"	35	5	+45	-175	-45	-20	Died 2 days
11	"	35	1	-45	-70	-45	+1	Lived
12	"	35	1	-30	-20	-40	-	Died 2 days
13	3% sodium bicarbonate	25	5	+50	-2	-2	-60	Died 3 days
14	"	25		-30	-120	-50	-100	Died 3 days
15	"	30	1	+25	+125	+10	-285	Died
16	"	30	1	-30	+30	-2	-920	Died
17	"	30	2	+10				Died
18	"	35	3	+5	+1			Died
19	Buffered dist water	25	2	+100				Died
20	"	25	5	+5	-25	-60	-110	Died 3 days
21	"	30	1	+160	-180	-50	-130	Lived
22	"	30	5	-60	-35	-120	-20	Lived
23	"	35		+10	-6	-4	-17	Died 4 days
24	"	35	1	-60	-110	-220	-10	Died

The results are given in Table IV. The experiment fails to demonstrate any appreciable difference in toxicity of neutral acryflavine in the three solvents for normal rabbits. It is the practice to inject neutral acryflavine in physiologic sodium chloride. Such solutions are acid. The other two solvents used in this experiment are more alkaline. Neutral acryflavine solutions prepared with these latter solvents apparently have equal bactericidal action and equal toxicity for normal rabbits to that of solutions prepared with physiologic sodium chloride. We do not know the comparative toxicity for rabbits suffering with infectious acidosis. The more alkaline solutions may be more desirable in such cases. The value of sodium bicarbonate remains to be

demonstrated While these experiments failed to demonstrate the value of sodium bicarbonate there is much to support the idea that it should be beneficial in the dye therapy of septicemia

It should be noted that this experiment does not indicate the maximum nonlethal dose or the lethal dose We used repeated doses rather than single doses Our rabbits were normal Animals suffering with septicemia are probably less resistant to the dye The practice of using not more than 7 to 11 mg per kilo in infections may be justified A normal cow gave symptoms of severe shock following one injection of 11 mg per kilo The rabbits lost weight following most of the dye injections All had diarrhea Rabbits usually lose weight following the injection of much smaller amounts of the dye Such rabbits ordinarily recover

This experiment demonstrates that normal rabbits vary greatly in resistance to intravenous injections of neutral acriflavine An occasional rabbit will live after five daily injections of 30 and 35 mg per kilo of body weight Our average rabbits resisted four daily injections of 20 mg per kilo

CONCLUSIONS

1 The P_H of neutral acriflavine solutions varies with the solvent and undoubtedly varies greatly as applied clinically Variations in P_H may account for some of the unfavorable results obtained clinically

2 The percentage of acriflavine going into solution varies with the solvent

3 The stability of acriflavine solutions varies with the solvent Only freshly prepared solutions should be injected intravenously

4 The dye solvent may be materially modified without greatly affecting the bactericidal action of the dye solution The possibility of combining with the dye some substance, such as dextrose, that will support the patient without reducing the effect of the dye on the bacteria deserves serious consideration

5 The best solvent for neutral acriflavine for intravenous therapy is not known Physiologic saline may be used with acriflavine but not with gentian violet Tap water, because it is more likely to be alkaline, may be a better solvent than distilled water We believe a buffered physiologic saline made from an alkaline tap water more desirable than physiologic saline made from distilled water The buffer salts should be added before the sodium chloride The dye should be added to the warm solvent As soon as the dye is dissolved the solution should be filtered and injected without allowing it to cool below body temperature If ordinary precautions are observed, sterilization by heat is unnecessary It may be harmful A solution in which precipitation has occurred should not be used

6 Normal rabbits vary greatly in ability to resist intravenous injections of neutral acriflavine Clinical records indicate a similar variation in resistance among human patients Since normal animals vary it may be assumed that all the variation shown by patients is not due to the type and stage of the infection but that individual variations exist What is urgently needed

is some method for detecting those particularly susceptible to the dye. Such patients should not be injected or should receive smaller doses than the average patient can resist.

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THE EFFECT OF INTRAVENOUS INJECTIONS OF NEUTRAL ACRIFLAVINE ON THE BACTERIOSTATIC ACTION OF THE BLOOD*†

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IN THE chemotherapy of septicemia it is desirable to know whether the therapeutic agent used is effective as the result of acting on the bacteria or on the host. If it acts as an antiseptic the smaller the number of bacteria in the blood stream the better the chance for beneficial results. Mass action is a factor in the sterilization of the blood stream as well as in the sterilization of other fluids. Ordinarily in the early stages of a progressing infection the number of organisms is smallest and the bacteriostatic agent should be injected as soon as possible. This will be true if the immune bodies play no part. However, if the destruction of the organisms is determined by the combined action of the therapeutic agent and the immune bodies, it does not follow that the earlier the injection the better the chance for cure. The immune bodies are more likely to be present and effective in the later stages of the disease. Furthermore, if the therapeutic agent acts by stimulating the mobilization of immune bodies rather than by acting directly on the bacteria the injection should be made when the mobilization of immune bodies is most likely to take place and be effective. This may not always be in the early stages of the disease.

Raiziss, Severae, and Moetsch¹ found that animals infected with staphylococcus died before the controls when the dyestuff was injected from one to three hours after infection but that beneficial results were obtained if the injection was delayed for forty eight to seventy two hours. They suggest that the beneficial effect of the late injection may have been due to the action of the dyestuff on the host resulting in a mobilization of the immune bodies when these were more effective than in the early stages of the infection.

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A second explanation of the results obtained by Raiziss, Severac, and Moetsch, and one that appears to us to harmonize better with what we know of the action of dyestuffs on the body and on bacteria, is that the dye in tolerated dose has weak antiseptic action on the bacteria. It alone in the early stages of the infection is insufficient to destroy the organisms. But that after the infection has progressed for some time and mobilization of immune bodies has occurred as the result of the infection the combined action of the dye and immune bodies is sufficient to check the infection.

If this is true then we have an explanation of the divergent results obtained both clinically and experimentally. Thus we can state that dye therapy in septicemia appears to be beneficial in those patients who tolerate the dye well if it is injected after the mobilization of immune bodies has begun. If there are no immune bodies the infection may not be materially checked. If there are sufficient immune bodies the patient will recover without the dye. If the patient is particularly susceptible to the dye the injection may be disastrous. Under such circumstances when to inject becomes a serious problem.

The question of whether the dyes in the blood stream act as an antiseptic or on the host is worthy of further discussion and experimentation. The evidence is not altogether conclusive. The clinical evidence, which is evidence obtained without adequate controls, is conflicting as to the value of the dyes intravenously, and how they act on the bacteria. Experimental work on laboratory animals although properly controlled is likewise confusing. The effect of the dyes on the bacteriostatic action of the blood, while attendant with more difficulties than appear on the surface is more readily and accurately determined than the therapeutic value.

Evidence concerning the effect on the bacteriostatic action of the blood of well-tolerated doses of acriflavine is not favorable. Spencer² failed to detect any bactericidal action in rabbit blood following injection of a tolerated dose. Burke and Newton³ found no increase in the bacteriostatic action of rabbit blood for staphylococcus following the injection of 11 mg per kilo of body weight. Melenev and Zau⁴ detected some bacteriostatic action for streptococcus after the injection of 25 mg per kilo. No effect was noted following the injection of nonlethal doses of acriflavine. After the injection of 125 mg per kilo the kidney tissue and after 25 mg per kilo both kidney and liver tissue inhibited streptococcus.

It is obvious that if an antiseptic in amounts tolerated by the average individual increases the bacteriostatic action of the blood, that antiseptic has therapeutic value. There still remains the necessity of determining under what conditions it should be used. If used under any and all conditions of septicemia conflicting results will be obtained. Since the results obtained with neutral acriflavine are conflicting it seems advisable to investigate further the effect of intravenous injections of this dye on the bacteriostatic action of the blood. The following experiments were designed with that object in view.

Experiment 1—To determine the bacteriostatic action of rabbit blood following the intravenous injection of neutral acriflavine.

One per cent neutral acriflavine solutions were freshly prepared in physiologic sodium chloride, in 3 per cent sodium bicarbonate, and in buffered distilled water. Amounts of each from 15 to 35 mg per kilo were injected intravenously in rabbits. Ten to fifteen minutes later blood was drawn from the heart. This blood was added in amounts from 5 to 40 drops, to tubes containing 10 cc of 2 per cent agar. The agar was poured into the half of a Petri dish. The other half of the Petri dish contained dye free blood agar. Strokes of *Staphylococcus albus* and *Bacterium coli* were made across both halves of the dish. The results are given in Table I. The experiment was repeated three times with comparable results. There was a very definite bacteriostatic action in the blood of rabbits receiving 35 mg per kilo of neutral acriflavine. This action was demonstrated for *Staphylococcus albus* but not for *Bacterium coli*. *Bacterium coli* grew on both halves of the dish but *Staphylococcus albus* grew only on the dye free blood agar. Five drops of blood from a rabbit receiving 35 mg per kilo in 10 cc of agar inhibited growth of *Staphylococcus albus*. Forty drops of blood from a rabbit receiving 30 mg per kilo delayed growth of the organisms for five days but did not entirely inhibit it. Twenty drops delayed growth for three days. The

TABLE I

RESULTS OF EXPERIMENT I TO DETERMINE THE BACTERIOSTATIC ACTION OF RABBIT BLOOD FOLLOWING THE INTRAVEINOUS INJECTION OF NEUTRAL ACRIFLAVINE

NO DROPS OF BLOOD IN AGAR	BACTERIUM COLI MG PER KO					STAPHYLOCOCCUS ALBUS MG PER KO				
	15	20	25	30	35	15	20	25	30	35
<i>5% Sodium bicarbonate dye solution</i>										
5	+	+	+	+	+	+	+	+	+	-#
10	+	+	+	+	+	+	+	+	+	-
15	+	+	+	+	+	+	+	+	+	-
20	+	+	+	+	+	+	+	+	+-	-
25	+	+	+	+	+	+	+	+	+	-
30	+	+	+	+	+	+	+	+	+	-
35	+	+	+	+	+	+	+	+	+	-
40	+	+	+	+	+	+	+	+	+-	-
<i>Physiologic sodium chloride dye solution</i>										
5	+	+	+	+	+	+	+	+	+	-
10	+	+	+	+	+	+	+	+	+	-
15	+	+	+	+	+	+	+	+	+	-
20	+	+	+	+	+	+	+	+	+-	-
25	+	+	+	+	+	+	+	+	+	-
30	+	+	+	+	+	+	+	+	+	-
35	+	+	+	+	+	+	+	+	+	-
40	+	+	+	+	+	+	+	+	+-	-
<i>Buffered distilled water dye solution</i>										
5	+	+	+	+	+	+	+	+	+	-
10	+	+	+	+	+	+	+	+	+	-
15	+	+	+	+	+	+	+	+	+	-
20	+	+	+	+	+	+	+	+	+-	-
25	+	+	+	+	+	+	+	+	+	-
30	+	+	+	+	+	+	+	+	+	-
35	+	+	+	+	+	+	+	+	+	-
40	+	+	+	+	+	+	+	+	+-	-

The plus sign indicates growth

+- The plus minus sign indicates growth was delayed for several days

The minus sign indicates no growth

minimum amount of dye that will increase the bacteriostatic action of the blood remains to be determined. Whether this amount can be tolerated by man is not definitely known. Clinical reports indicate that it can. In this connection it should be noted that the bacteriostatic action of the acriflavine-containing blood was demonstrated in the presence of agar. Agar greatly reduces the action of the dye and also of the blood. Dyes are more effective in a liquid medium. It is difficult to detect a slightly increased bacteriostatic action in acriflavine-containing blood. Since blood is not transparent counting the organisms must be done. The factors of dormancy and increased tolerance of the organisms for the dye interfere with the detection of slight bacteriostasis by counting methods. Burke and Newton tried this method with gentian violet with unsatisfactory results.³ Stroking the surface of heat-coagulated acriflavine-containing blood is also unsatisfactory as the heat affects the natural antibodies. We tried this method and found that *Staphylococcus albus* failed to grow on the surface of blood heated to 60° C for one hour when taken from a rabbit receiving 30 mg per kilo but grew vigorously on the blood taken from a rabbit receiving 20 mg per kilo. The organisms were inhibited but not killed. Growth occurred on blood from a control rabbit. Certain factors, such as growth lag and acquired tolerance for the dye, interfere with the determination of slightly increased bacteriostatic action in whole blood. Since we demonstrated increased bacteriostatic action in the blood of rabbits in agar and in heat-coagulated blood following the injection of 30 mg per kilo, it is safe to assume that the injection of smaller amounts of dye will increase the bacteriostatic action of the blood in the normal rabbit for *Staphylococcus aureus*. Since this increased action is very temporary, all we can hope for is to shock the bacterial cells sufficiently to enable the body defenses to destroy them. The evidence bearing on whether this actually occurs, or under what conditions it occurs, is conflicting. The dye as applied clinically apparently turns the tide in favor of the patient in some cases and against him in others. Many factors affect the result. These are largely ignored.

The experiment does not demonstrate bactericidal action in the dye-blood agar. The *Staphylococcus albus* organisms were inhibited but not killed. A loop drawn along the stroke on the dye-blood agar was streaked on fresh agar. Vigorous growth resulted. Five cubic centimeters of blood drawn from the heart of a rabbit ten minutes after the injection of 30 mg per kilo of neutral acriflavine in buffered distilled water was inoculated with 0.5 cc of a suspension of *Staphylococcus albus*. The mixture was incubated twenty-four hours. Transfer to agar slants resulted in vigorous growth. Apparently the blood of rabbits does not become bactericidal following the injection of that amount of neutral acriflavine.

In the previous experiment tests of the bacteriostatic and bactericidal action were made on blood drawn from the heart within a few minutes after the injection of the dye. The experiment demonstrated bacteriostatic but not bactericidal action. The following two experiments were designed to determine whether there is an increase in bactericidal action in the body of a normal rabbit following the injection of a large dose of neutral acriflavine.

In Experiment 2 the rabbit was killed shortly after the injection of the dye to insure the dye remaining in the blood stream. Dyes are excreted in a few hours. In Experiment 3 the rabbit was not killed for twenty four hours. In this rabbit the organisms were exposed to the dye in the blood stream for only a short time. Experiment 4 was designed to determine whether the dye had sufficient effect on the cocci to delay the infection and retard death. Sufficient experimental evidence has been presented recently to suggest that the injection of the dye might hasten death.

Experiment 2—To determine whether *Staphylococcus aureus* will be destroyed in the body of a rabbit killed immediately after the injection of a large dose of neutral acriflavine.

Two rabbits were injected intravenously with 2 cc of a twenty four hour suspension of *Staphylococcus aureus*. Ten minutes later 1 rabbit was killed and placed in the incubator for twenty four hours. The second rabbit received 35 mg per kilo of neutral acriflavine in buffered physiologic sodium chloride. Ten minutes later this rabbit was killed and incubated for twenty four hours. Both rabbits were autopsied and cultures made from kidney and liver were incubated. Luxuriant cultures of *Staphylococcus aureus* resulted. The experiment failed to demonstrate any bactericidal action in the body of the rabbit receiving the dye. Since the normal defensive mechanism is largely reduced at death it seemed advisable to test for bactericidal action in the living rabbit.

Experiment 3—To determine whether *Staphylococcus aureus* will be destroyed in the body of a normal rabbit following the administration of a large dose of neutral acriflavine.

Two rabbits were injected intravenously with 2 cc of a suspension of *Staphylococcus aureus*. Twenty minutes later one of these rabbits received 35 mg per kilo of neutral acriflavine in buffered distilled water. Twenty four hours later both rabbits were killed and cultures made from liver, spleen, and kidney. *Staphylococcus aureus* was recovered in all cases. The experiment failed to demonstrate any bactericidal action in the rabbit receiving the dye. It also failed to demonstrate that the cocci were sufficiently injured by the exposure to the dye during its short period of circulation in the blood stream to enable the natural defensive mechanism to destroy them in twenty four hours. It should be noted that 2 cc of a suspension of a twenty four hour culture is a heavy dose. The result might have been different if a smaller dose had been given.

Since our previous experiments had demonstrated an increase in the bacteriostatic action of rabbit blood following the injection of 35 mg per kilo of neutral acriflavine, it seemed desirable to determine whether this action was sufficient to check a *staphylococcus* infection.

Experiment 4—To determine whether the bacteriostatic action of neutral acriflavine in the blood of a rabbit is sufficient to check an infection with *Staphylococcus aureus*.

Three rabbits were injected intravenously with 2 cc of a heavy suspension of a virulent culture of *Staphylococcus aureus*. Twenty four hours

later all the rabbits showed severe symptoms and to the same degree. At this time rabbit No 1 was given 35 mg per kilo of neutral acriflavine in buffered physiologic sodium chloride. There was marked improvement in two and a half hours after the injection of the dye and in four hours the animal was breathing normally and eating grass. The animal died six days after the injection of the dye. There were typical staphylococcus lesions in kidney, liver, and heart. Rabbit No 2 was given 4 injections of the same dye solution of 9 mg per kilo at six-hour intervals. This rabbit received approximately the same amount of dye per kilo as rabbit No 1 but in multiple doses. The object was to determine whether multiple doses would prove as effective as one large dose. Bacteriostatic action in the blood has never been demonstrated following the injection of 9 mg per kilo. Approximately this amount has been used clinically. This rabbit did not show the marked improvement of rabbit No 1 and died three days after the dye injections were begun. Staphylococcus aureus was recovered from the organs. Rabbit No 3 served as a control. It died within twenty-four hours after rabbits Nos 1 and 2 began receiving dye injections. Numerous other controls indicated that death was to be expected at this time.

The results obtained indicate that under certain conditions Staphylococcus aureus infections in rabbits can be checked by the intravenous injections of neutral acriflavine. Apparently one large dose is more effective than the same amount of the dye in multiple doses. Our rabbits died, but it should be noted that the progress of the infection was checked even though the dosage of organisms was excessive. It is possible that the animals may have been completely protected from smaller doses. Mass action is an important factor and one frequently overlooked in experimental work. As demonstrated by Kolmer, Schamberg, and Raiziss a certain amount of disinfectant will lead to sterilization of the blood of an animal when inoculated with a restricted number of parasites but will not do so when the number of parasites is greatly increased.⁵

DISCUSSION

The value of neutral acriflavine in septicemia has recently been questioned by Meleney and Zan.⁴ They made extensive and well-controlled investigation into the effect of neutral acriflavine on the blood cells, tissues, and on the bacteriostatic action of the blood. They failed to find an increase in the bacteriostatic action of the blood for streptococci following the injection of nonlethal doses of acriflavine. There was bacteriostatic action in some cases following the injection of 25 mg per kilo. The injection of nonlethal doses in rabbits suffering with hemolytic streptococcus infections hastened death. They conclude that in so far as hemolytic streptococcus infections are concerned neutral acriflavine is not a legitimate intravenous medicament.

Our results differ from those of Meleney and Zan in two important respects. Our normal rabbits withstood much larger amounts of neutral acriflavine. We do not know whether this was due to a different method of preparing the dye solution or to a different make of dye. This suggests

that either the method of preparation of the dye solution or the methods of manufacture are not sufficiently standardized to produce the best results. Some of the conflicting reports can be accounted for on this basis. Secondly, the injection of the dye in our rabbits suffering with an infection did not hasten death but prolonged life. Our rabbits were suffering with a staphylococcus infection and those of Melnick and Zan with a hemolytic streptococcus infection.

It is evident that the experimental work on neutral acriflavine so far described is too limited and conflicting to serve as a basis for adequately estimating the dosage and value of the dye for intravenous therapy. The nature of the conflicting experimental evidence has been presented by Eggerth and need not be repeated here.

Clinical reports indicate that in some hopeless cases the injection of the dye apparently turns the tide in favor of the patient and at other times against him. We should not expect favorable results in all cases. Patients apparently vary in tolerance for the dyes. As our knowledge increases we may be able to select with greater certainty those cases in which intravenous dye therapy is indicated. As Hanzlicz has pointed out there is danger associated with all intravenous medication.⁷ But in hopeless cases this danger may be a legitimate risk.

All we should hope for or expect in the use of neutral acriflavine in the treatment of septicemia is a slight shock or check to the invading organisms. The dye cannot be used in sufficient strength to kill the organism. The brief exposure to the diluted dye may check them. The body must do the rest. So many factors are operating that we should expect variable and sometimes disastrous results. Some of the factors are subject to control and can be eliminated. It is the function of the experimenter to determine what these are and to eliminate them. A partial list of the variants which affect the results and deserve consideration are: type of organism, number of organisms, variation in virulence, variation in resistance of host to dye and to the organisms, resistance of the organisms to the dye, hydrogen ion concentration of the solution, variation in the manufacture of the dye and in the preparation of the dye solution and in its application. The type of infection is important. Success is less certain in those cases in which there are local abscesses discharging fresh showers of organisms into the blood stream. It is thus seen that variations in the host, in the parasite and in the dye solution deserve consideration before the value of neutral acriflavine as an intravenous antiseptic can be properly determined. Furthermore we venture the opinion that progress in intravenous therapy will be more rapid when more attention is paid to combining a number of compatible agents, each slightly aiding the patient and reducing shock rather than as is done at present, depending on the injection of a single agent. The addition of glucose may be beneficial.

The dyes are not perfect blood antiseptics but they are serviceable and offer the best treatment available under some conditions, and their use is justified until something better replaces them. At present it is impossible to state which of the antiseptic dyes will give the best results under a given set

of conditions. Some of our experiments, to be published, indicate that gentian violet, although more toxic, should in general prove more effective than acriflavine.

CONCLUSIONS

1 The intravenous injection of 30 mg per kilo of neutral acriflavine in the normal rabbit causes an increase in the bacteriostatic action of the blood for *Staphylococcus albus*. No increased action for *Bacterium coli* was demonstrated. The minimum dose that will bring about an increased bacteriostatic action was not determined. The technic used by us failed to indicate an increase in the bacteriostatic action of the blood following the injection of less than 30 mg per kilo.

2 The intravenous injection of 35 mg per kilo does not cause the blood to become bactericidal for *Staphylococcus aureus*.

3 The intravenous injection of an excessive dose of neutral acriflavine does not always hasten the death of a rabbit with a fatal infection of *Staphylococcus aureus*. It will in some cases delay death.

4 The clinical and experimental evidence as to the value of acriflavine in septicemia is very conflicting. The factors affecting the value of the dye are given in the discussion.

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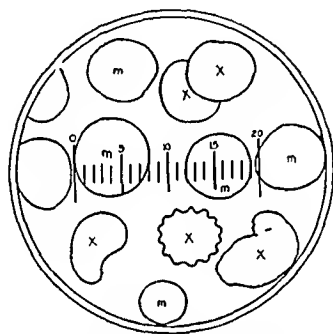
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A STUDY OF ERYTHROCYTE DIAMETERS IN THE NEWBORN*

BY HERBERT SILVETTE RICHMOND, VIRGINIA

SEVERAL observers^{1 2 3 4} have published reports on the mean diameter and the variability of the erythrocytes of the normal adult, as well as the changes noted in the size of the red cells in the various anemias. These reports naturally led to the question as to whether or not the red corpuscles of the normal newborn child differed in size and variability from the figures determined for the normal adult.

For this purpose only the erythrocytes of normal, healthy babies were measured. The blood was obtained as soon as possible after the time of birth and in no case after forty eight hours. The average age of the babies in the



m = type of cell measured
X = type of cell not measured

Fig 1—Diagram showing micrometer eye piece in position to measure the red cells

series was twenty five hours. The 30 cases in the group represent both males and females, no attention was given the sexes of the subjects in the study.

Blood slides were made by the two slide method since it is easily possible in this way to get smears which are thin enough to separate the cells and at the same time avoid the distortion due to too great spreading. Only those cells which retained their natural round or oval shape were measured, those distorted or ruptured by mechanical means as well as those which were overlapping, were passed over. The diameters of the erythrocytes were measured by means of an oil immersion objective together with an 8x micrometer eye piece standardized with a Levy Neubauer counting chamber. Fig 1 shows

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the method of determining the diameters of some representative cells. The diameters of 200 cells were measured in each case, giving a total of 6,000 cells for the series. The mean diameter of 200 cells measured in each case was taken as the average diameter of the erythrocytes of that particular baby.

The following formula was worked out and used to calculate the mean diameter in each case

$$\text{Mean Diameter} = \frac{N + N' + N'' + \dots}{D \times N + D' \times N' + D'' \times N'' + \dots}$$

where D = a certain diameter, for instance, 56μ

N = the number of cells of that diameter, i.e., 9

D' = another diameter, for instance, 64μ

N' = the number of cells of that diameter, i.e., 17

At the same time that the mean diameters were determined, it was decided to make a red blood count and a hemoglobin estimation on each case.

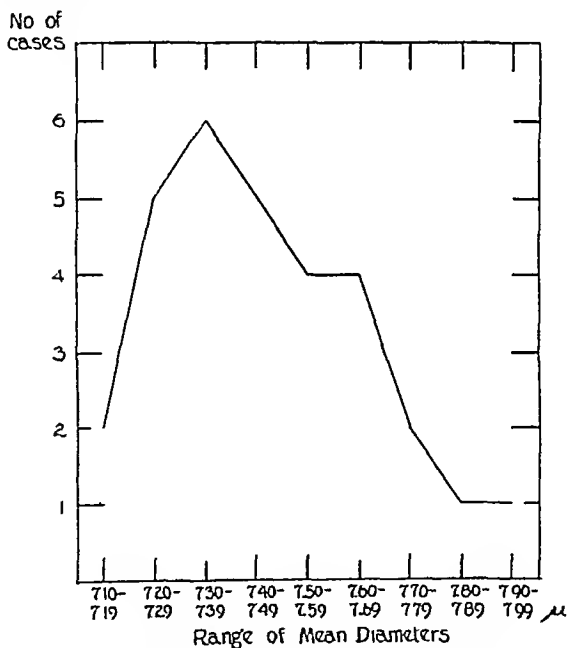


Fig. 2—Chart showing the distribution of 30 mean diameters between 717 and 796 microns

and so to calculate the average color index of the blood of the newborn child. For this purpose, the Newcomer hemoglobinometer, approximately accurate to 1 per cent, was used.

Of the 30 cases, the mean diameters ranged from 717 to 796 microns, with an average mean diameter for the whole series of 745μ . Fig. 2 is a graph showing the distribution of the mean diameters between the above extremes. The greatest number of mean diameters are massed so as to form a peak very near the average diameter of the whole group. Of the 6,000 cells measured, 17 were 40μ in diameter, 47 were 48μ , 169 were 56μ , 632 were 64μ , 2400 were 72μ , 2391 were 80μ , 261 were 88μ , 74 were 96μ , and 9 were 104μ . The curve plotted using the number of cells measured and their diam-

eters (Fig 3) is typical in that by far the greatest number of cells form a sharp peak between 7.2 and 8.0 μ , as nearly as it is possible to ascertain with a micrometer ocular. The largest cells measured were 10.4 μ in diameter, while the smallest were 4.0 μ . The difference in size between the largest cell and the smallest in each case—the variability—ranged between 3.2 and 6.4 μ . The average variability of the 30 cases was 4.88 μ .

The following gives, in addition to the figures above, the different blood elements determined along with the mean diameter and the variability of the cells.

Number of babies studied 30

Number of erythrocytes measured 6 000

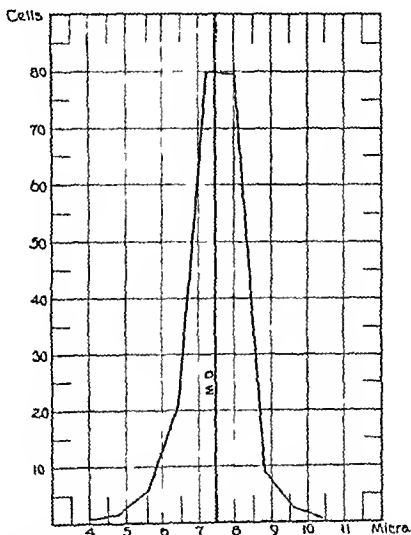


Fig 3—Composite chart of red cell diameters of 20 babies 6000 cells measured chart reduced to scale of 80 cells MD=mean diameter

Age of babies, from four to forty eight hours, average age, twenty five hours

Mean diameters, from 7.17 to 7.96 μ average mean diameter, 7.45 μ

Variability, from 3.2 to 6.4 μ , average variability, 4.88 μ .

Red blood counts, from 3,280,000 to 5,700,000, average red blood count 4,519,000

Hemoglobin, from 71 to 110 per cent average hemoglobin, 92.9 per cent (average of 20)

Color index, from 0.86 to 1.16, average color index, 1.02

As the color index is usually high when the size of the cells is increased as in pernicious anemia, and low when the mean diameter is decreased as in secondary anemia, it was thought that the two figures would parallel each

other in the case of the babies studied. In a general way this was so in the individual cases, and the averages for the group showed this correlation very well. The mean diameter at 7.45 micra and the color index at 1.02 for normal newborn babies ran parallel to but somewhat higher than the corresponding figures of 7.21¹ μ and 1.00 for the normal adult. In fact, $7.21 : 7.45 = 1.00 : 1.02$ is a fairly true proportion.

Fig. 4 is included as a typical chart of the diameters and the variability of the red corpuscles of a normal baby.

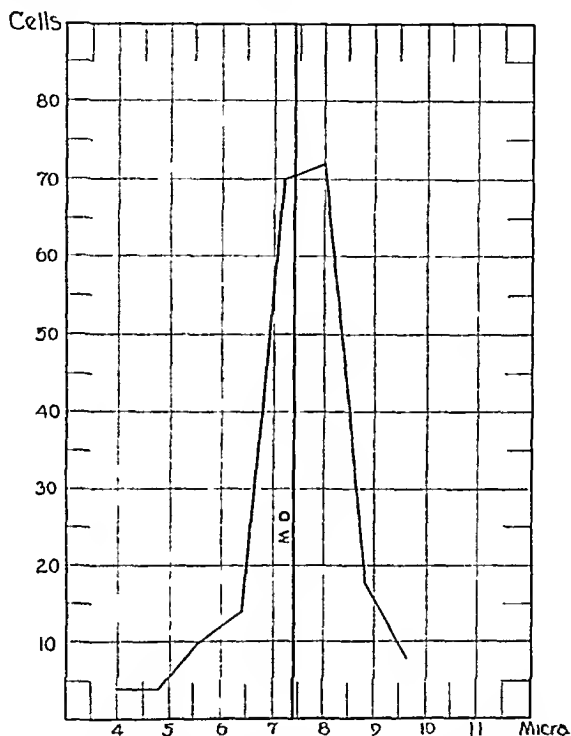


Fig. 4—Chart of red cell diameters of a typical case. MD—7.38 micra. Variability—5.6 micra. R.B.C.—4,250,000. Hb. 86 per cent. 18 hours old. 200 cells measured.

Both the diameters of the erythrocytes themselves and the degree with which they vary in size is very constant, and in these respects the red blood cells of the normal newborn child exhibit the same remarkable constancy as do those of the adult.

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NOTE ON SACCHAROMYCES MALI DUCLAUXI ISOLATED FROM A THROAT CULTURE*

By RACHEL L. HOFFSTADT PH.D. D.Sc., SEATTLE, WASH. †

THE existence of membranes in the throats of persons, other than those caused by the diphtheria organisms has frequently been observed. They vary in color, consistency, and persistence. *Oidium albicans* is often found to be the cause of such membranes.

Grover¹ (1916) cultured seventeen varieties from diphtheria cases and diphtheria contacts but named none of them.

Ruth, aged twelve, a healthy child, showing no temperature or other symptoms, developed a persistent white flaky membrane on the left tonsil. The case was diagnosed from the type of membrane, and on microscopic examination, as *Oidium albicans*. The organism, when cultured on glucose agar, grew abundantly in a moist raised colony, while on agar agar the growth was slow and scant. In broth a sediment was first formed which was followed by the formation of a light, floating scum and "ring" on the side of the tubes. As the cultures aged the ring in some instances, reached the top of the tube, giving the tube the appearance of being etched.

It fermented glucose broth with the formation of gas and acid, and glyceric broth, with acid. It did not ferment lactose, sucrose, maltose, inulin, or salicin broths. On potato it formed a moist growth which, after seventy-two hours, extended to the sides of the test tubes opposite the slant. No change was formed in milk. A scanty growth along the line of puncture was seen in gelatin. These cultural reactions agree with the organism two described by Grover.

The yeast cells in young cultures were 6 microns in length and 3 microns in width. They showed little vacuolation when young but became highly vacuolated at the end of forty-eight hours. On solid medium and in the sedimentary deposit of old liquid medium they formed a septate mycelium of from 3 to 11 cells. Each cell was from 1 to $1\frac{1}{2}$ times the length of a parent yeast cell. At this point it might well be mistaken for an *Oidium albicans*.

The cells germinated by budding. After seventy-two hours at 20° C. ascospores are formed in the cells in the scum and on the sides of the glucose broth tubes. During sporulation the cytoplasm becomes highly vacuolated. Regularly 16 basophilic granules appeared and were most frequently placed around the edge of the cell. These arranged themselves in groups of eight at the ends of the cell before the formation of the two ascospores. No more than two ascospores were observed. The spores were surrounded by a single

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membrane No trace of copulation was seen, but the regular arrangement of the basophile granules might indicate rudimentary sexual phenomena The ascospore germinated in a single direction

The thermal death point was 55° C The organism was not pathogenic to guinea pigs

This yeast agrees in description with that isolated by Kayser² from cider, *Saccharomyces mali* Duclaux He found it imparted the bouquet to the solution

Saccharomyces mali Duclaux resembles *Oidium albicans* in morphology during its yeast-like form, and might be mistaken for it on microscopic examination It differs from it in that it grows more abundantly on ordinary culture media and ferments glucose with the formation of gas and acid and glycerin, with acid The type of membrane formed on the throat by *Saccharomyces mali* Duclaux was similar in appearance to that formed by *Oidium albicans*, but the patient from whom it was isolated showed no temperature or other symptoms

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LABORATORY METHODS

THE EVALUATION OF BRIGGS METHOD FOR THE COLORIMETRIC DETERMINATION OF PHOSPHORUS*

By HELLEN B. BENNETT, B.A. NEW HAVEN, CONN.

THE colorimetric determination of small amounts of phosphorus according to Bell and Doisy,¹ as modified by Briggs,² has been widely and successfully used for the determination of phosphorus in blood. The principle of the method depends upon the formation of phosphomolybdate which is reduced by hydroquinone to give a blue color proportional to the amount of phosphorus present. When this method is applied to physiologic material other than blood, such as food, urine and feces, the accuracy of the method is still open to investigation. The present study is an attempt to evaluate the optimal conditions of the concentration of reagents, the sulphuric acid concentration, and the salt concentration upon the reduction of phosphomolybdate by hydroquinone, as measured by the blue color developed.

EXPERIMENTAL

The procedure for color development recommended by Briggs in his blood method² has been designated the "standard" procedure with which our subsequent determinations have been compared. All the experiments were conducted upon standard solutions of monopotassium phosphate. The usual sodium molybdate, hydroquinone, and sodium sulphite reagents were used. The rate of color production under "standard" conditions is expressed in per cent of that developed in twenty four hours (Fig 1). The data show that at thirty minutes the color developed is within 5 per cent of that obtained at the end of an hour and that a five minute difference in the time of reading causes only a 1 per cent error. At one hour and subsequently, the rate of change is so slow that hourly differences in time do not matter. In from one to five hours 90 per cent of the color value developed in twenty four hours is obtained.

THE CONCENTRATION OF REAGENTS

The effect of developing the color in a more dilute solution than that specified under the "standard" procedure upon the proportionality of phosphorus has been determined. The limits of the amount of phosphorus which the method can handle have not been adequately defined in the literature. At least 0.2 mg per cent of phosphorus must be present in order to obtain a color deep enough to read in the colorimeter. The color obtained with a phos

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phorus content above 20 mg per cent is too deep to read accurately without dilution. The limits of proportionality of the color with the phosphorus content were determined with various final volumes at forty minutes. The amount of reagents were constant throughout. Table I expresses the amount of phosphorus in each solution calculated from the intensity of color relative to that of the others in the same series. Solutions which had a greater phosphorus content than 20 mg per cent were diluted just before being read. This table shows that proportionality is maintained from 0.2 mg to 30 mg per cent under "standard" conditions (Column 1). With two and a half times the "standard" volume, proportionality is kept from 0.2 mg per cent to 20 mg per cent (Column 2). In five times the volume proportionality is maintained from 0.2 mg to 12 mg per cent (Column 3), while it is maintained from 0.2 mg to 0.5 mg per cent in ten times the volume. As proportionality is maintained within these defined limits, it is obvious that the rate

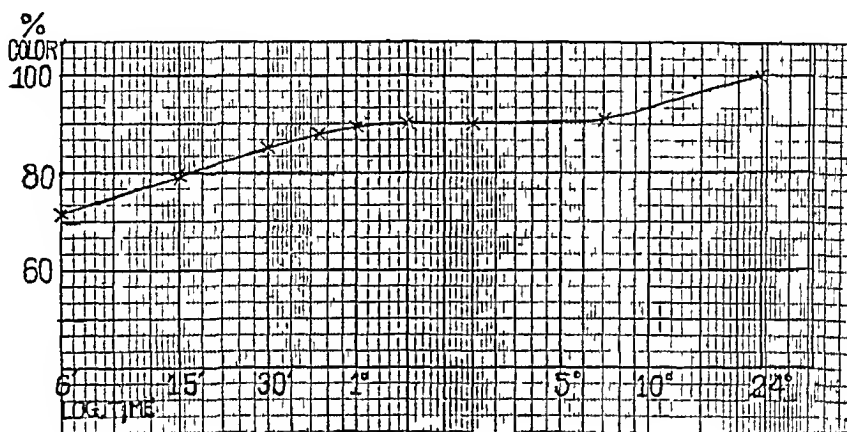


FIG. 1

of color development with different phosphorus concentrations is the same under the same conditions of reagents. Dilution after the color has been developed does not alter proportionality.

Because proportionality is maintained in the same dilution it cannot be assumed that the same amount of color is obtained with different concentrations of reagents. When the final volume, at the time of the addition of the reagents, is greater than two and a half times the "standard" volume, less color develops. When the reagents are diluted to five and ten times the "standard" concentrations 85 and 70 per cent respectively, of the "standard" color is obtained as a maximum. This is shown in Fig. 2 where the milligrams per cent of phosphorus present are plotted against the per cent of theoretic color obtained under "standard" conditions, calculated from comparison with 10 mg per cent of phosphorus at forty minutes. Twice the "standard" concentration of the reagents, i.e. one-half the volume does not increase the amount or the rate obtained up to two hours. When the reagents are present in concentrations three and four times that of the "standard" the color and rate are not increased but an undesirable greenish tinge is present which

TABLE I

LIMITS OF PROPORTIONALITY OF PHOSPHORUS IN RELATION TO DILUTION OF REAGENTS

10 CC VOL RECOVERED MG PER CENT	25 CC VOL RECOVERED MG PER CENT	50 C VOL RECOVERED MG PER CENT	100 CC VOL RECOVERED MG PER CENT	P CONC PRESENT MG PER CENT
0.21	0.20	0.21	0.20	0.20
0.30		0.32	0.31	0.30
0.37	0.39	0.39	0.40*	0.40
0.50		0.51	0.48	0.50
0.58		0.61	0.53	0.60
0.70		0.70	0.52	0.70
0.78	0.80	0.80	0.51	0.80
0.93				0.90
1.00		1.00		1.00
1.20	1.20	1.20		1.20
1.40		1.20		1.40
1.60*	1.60	1.20		1.60
1.80		1.20		1.80
2.0	1.98	1.20		2.00
	2.00			2.20
2.30				2.40
				2.60
2.70				2.80
2.90				3.00
2.70				3.20
				3.40
				3.60
2.50				3.80
				4.00
Standard				

interferes with comparison. Hence, the concentration of reagents should be the same in the standard and in the unknown, and for optimal conditions should approach the 'standard' concentration. This error introduced by variations of conditions has been recognized by Bell and Doisy,¹ Stanford and Wheatley,⁴ and by Fiske and Subbarow.

The effect of changing the concentration of each reagent upon the color development was determined when the other reagents and the final acidity were maintained under "standard" conditions. It was found that the limits of the reagents at a final acid concentration of 0.25 M are as follows: molybdate, 0.5 to 1.0 per cent (10 to 20 cc of 5 per cent ammonium molybdate solution), hydroquinone, 0.05 to 0.25 per cent or more (5 to 10 cc of 0.5 per cent hydroquinone in 100 cc), sulphite, 0.2 to 2.0 per cent (1 to 10 cc of 20 per cent sodium sulphite solution in 100 cc). In agreement with Roe, Irish, and Boyd⁶ it was found that the molybdate reagent itself is reduced above the given limit. Excess sulphite prevents reduction of the phosphomolybdate.

THE SULPHURIC ACID CONCENTRATION

The effect of acid upon the color production was determined when the concentration of reagents was kept as defined under 'standard' conditions. The results obtained when the final acidity was varied from 0.05 M to 1.0 M are shown in Fig. 3. The maximum stable color is observed in acid concentrations of 0.05 M to 0.50 M from one to three hours. A final acid concentration of 0.25 M, which gives a stable color in from fifteen minutes to

twenty-four hours and which is the midpoint of this acid range, is, therefore, a safe acid concentration to use for a standard. This agrees with the conclusion of Stanford and Wheatley⁴ and Martland and Robison.⁷

THE SALT CONCENTRATION

The influence of salt upon color development is dependent upon the acidity, the concentration of reagents, and then proportion to each other in the final volume of the mixture. An investigation into the question could be indefinitely broad in its scope. An attempt has been made to determine the effect of some of the common salts which are formed on neutralizing the ash of physiologic material.

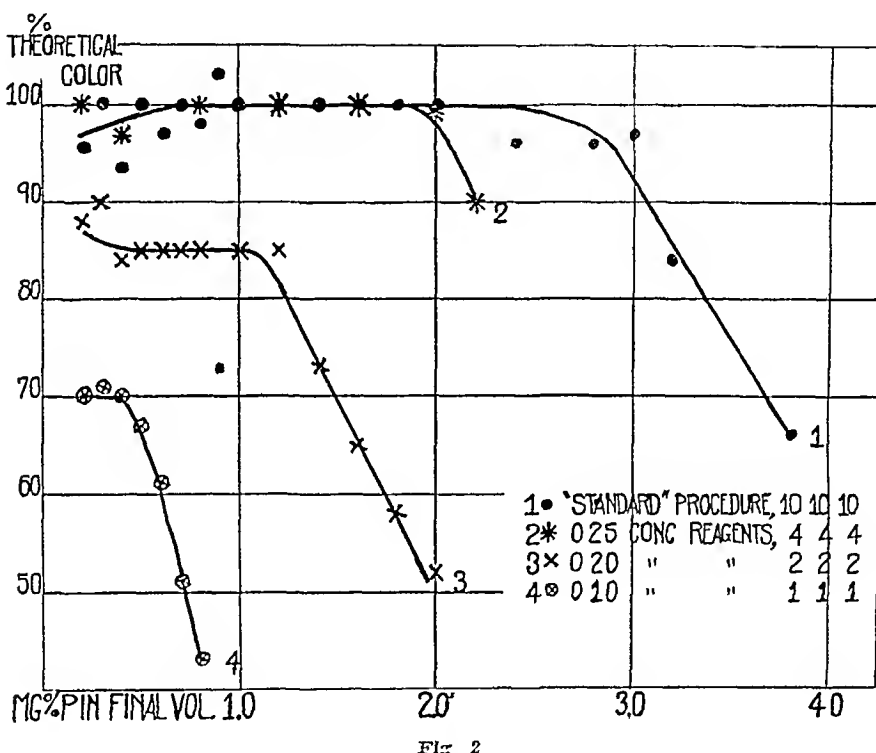


FIG. 2

The effect of salts upon the amount of color developed has been determined under "standard" conditions. These solutions were compared to a similar salt-free standard made up at the same time. The molarity of the salts has been defined in terms of their concentration in the colored solution. The salts studied in concentrations of from 0.2 to 1.0 M were sodium chloride, potassium chloride, sodium nitrate, ammonium nitrate, magnesium nitrate and sodium sulphate. The salt effects are small. The concentration where the salt effect is first noted differs for the various salts studied. The mono-mono salts (sodium chloride, potassium chloride, sodium nitrate, and ammonium nitrate) do not influence the color up to 0.6 M at thirty minutes. Sodium sulphate affects the color at 0.3 M and magnesium nitrate at 0.2 M. Several writers have recognized that salts

interfere with the color production^{3 5 *} In general, the effect is to increase the color at first and later to diminish it For practical purposes it can be said that when salts of unknown types and amounts are present it is best to compare the colors for from forty five minutes to one hour All the errors will not be avoided in this way, but when the reagents are present in proportion and amounts described under the "standard" procedure, the probable error may be assumed to be plus or minus 5 per cent

When the solutions are diluted five times the "standard," no augmentation of the color takes place during the early stages of color development There is a general retarding effect in some cases amounting to 70 per cent in fifteen minutes, which is marled even at low salt concentrations This does not affect the amount of color developed later for in nearly all cases normal values are obtained in three hours Hence, dilution of reagents should be avoided

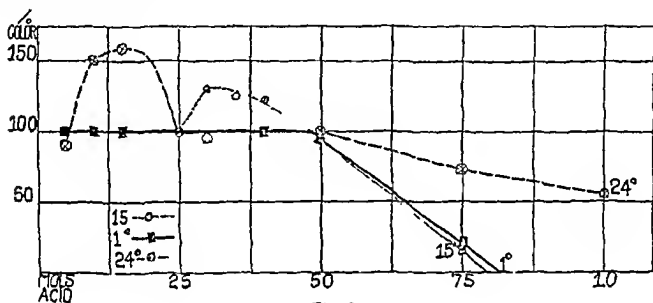


Fig 3

In order to ascertain the magnitude of error introduced by a combination of salts, a comparison between the usual gravimetric and colorimetric determinations was made upon urine and stools The errors are of the same order of magnitude as those obtained with individual salts The phosphorus determined in unashed urine with five times the volume of the "standard" at one hour gave errors from -35 per cent to +66 per cent In rat stools of low phosphorus content, when the reagents are diluted to two and a half times the "standard" or in the "standard" volume, an average error of plus or minus 5 per cent is obtained

The effect of salt concentrations when the acid is also present in varying amounts was determined in the case of sodium sulphate under "standard" conditions The solutions were compared with the salt free standard of the same acidity, developed simultaneously In the optimal acid range (0.05 M to 0.5 M) 0.01 M to 0.2 M sodium sulphate does not interfere Greater salt concentration (up to 1.0 M) causes an unstable color and depending upon the conditions either augments or decreases the rate of color development A 35 to 160 per cent increase in the color intensity is obtained with all concentrations of sodium sulphate used (0.01 to 1.0 M) in 0.75 M acid at all times

ADOPTED TECHNIC

The above results show that the conditions defined by Briggs for the determination of phosphorus in blood are correct. However, it is safer to change the amounts of the reagents to 15 cc of molybdate containing 16 m sulphuric acid, 15 cc of hydroquinone and 5 cc of sulphite per 100 cc final volume, or, keeping the convenient volume relationship of Briggs, to use the following reagents

TABLE II

A COMPARISON BETWEEN GRAVIMETRIC AND COLORIMETRIC PHOSPHORUS DETERMINATION OF STOOL ASH

HIGH P BRIGGS NEW					LOW P BRIGGS NEW					
*ORAY MG PER CENT	AUTHOR'S REAGENTS		BRIGGS' REAGENTS		*ORAY MG PER CENT	AUTHOR'S REAGENTS		BRIGGS' REAGENTS		
	MG PER CENT	ERROR PER CENT	MG PER CENT	ERROR PER CENT		MG PER CENT	ERROR PER CENT	MG PER CENT	ERROR PER CENT	
1 B	1.94	2.03	+ 5	2.0	+3	0.39	0.37	-5	0.37	-5
2 B	1.80	1.98	+10	1.89	+5	0.36	0.36	0	0.36	0
3 B	1.90	2.0	+ 5	1.92	+1	0.38	0.36	-5	0.37	-3
4 B	2.04	2.06	+ 1	2.0	-2	0.41	0.38	-7	0.38	-7
5 B	2.2			2.11	-4	0.44	0.41	-7	0.42	-4

*The amount of phosphorus present in the aliquot taken for the colorimetric determination is calculated from the gravimetric value.

Acid Molybdate Reagent —

37.5 gm of ammonium molybdate are dissolved in 300 cc of water and to this are added 200 cc of water containing 75 cc of concentrated C P sulphuric acid.

Hydroquinone Solution —

0.75 gm of hydroquinone is dissolved in distilled water and diluted to 100 cc. A drop of concentrated sulphuric acid is added as a preservative.

Sulphite Solution —

A 10 per cent solution of sodium sulphite.

When salts are present, the solution to be analyzed should be diluted if possible. An amount of phosphorus of 0.3 to 0.5 mg should be used and the color developed in 100 cc, using 10 cc of each of Briggs' reagents (or those given above). In this way, the proportionality of phosphorus is maintained, and the salt effect is diminished to a minimum. The colors should be compared at the end of an hour.

APPLICATION TO STOOL ASH

This technique, which was developed for pure salts, was tested on physiologic material. The solution of the ash of low phosphorus rat stools was determined gravimetrically as magnesium pyrophosphate, by Briggs' blood technique and by the new technique. The results given in Table II show fair agreement between the gravimetric and colorimetric methods. With small amounts of phosphorus negative errors up to 7 per cent resulted with both

colorimetric procedures. With a larger amount of phosphorus, positive errors (1 to 10 per cent) resulted with Biggs' reagents, but correct values (4 per cent) were obtained with the new reagents.

SUMMARY

1 Biggs' method for the determination of phosphorus has been studied in respect to the following factors

- a The rate of color development
- b The limits of phosphorus content
- c The limits of concentrations of reagents
- d The salt effects at various concentrations singly and combined and in the presence of various acid concentrations

2 Based on this study Biggs' reagents have been found to give correct but not optimal conditions and alternative reagents have been recommended.

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A NEW AND IMPROVED INJECTION APPARATUS*

By LOUIS GROSS M.D. New York City

THE study of vascular architecture and vascular disease processes by means of injection has assumed so much importance of late that it was thought advisable to describe an improved apparatus which simplifies and standardizes the technique to a point where it is practically foolproof.

The importance of a standardized uniform injection technique will be appreciated when it is realized that the old methods of injecting vessels by hand, using a syringe, can give no reliable comparative results inasmuch as it is impossible in this way to determine whether differences in the vascular tree are due to artefact or were present in the vessel structure itself.

Elsewhere, I described an injection cabinet which gave excellent results and permitted of fairly accurate standardization of all the technical factors concerned in injection of vascular channels.¹ This cabinet, however, demanded a certain amount of personal control and observation. In order to reduce the personal factor to a minimal degree the present apparatus was devised. By its use, extraordinarily beautiful and complete injections can be obtained and, since every step in the injection is automatically controlled and standardized, there is left very little room for error or personal equation.

¹ From the Laboratories of the Mount Sinai Hospital New York City
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Obviously the temperature of the pot (*R*) must be maintained at such a point as will deliver a current of washing fluid from (*Z*) at the desired temperature (usually 45° C). As indicated before, the fluid is conducted from the saline section into the injection section, through the bottle (*S*)

3 INJECTION SECTION

The injection section (Fig 3) consists of a wooden cabinet having a glass window (1) as a roof, and two doors which open in front. One door is large, the other small, the division between the doors being opposite the copper partition (2)

The entire cabinet is lined with copper, and is divided by a dwarf partition (2) into a smaller (3) and a larger chamber (4). The smaller chamber holds the rocker (5) of a shaking machine. The rocker is attached by means

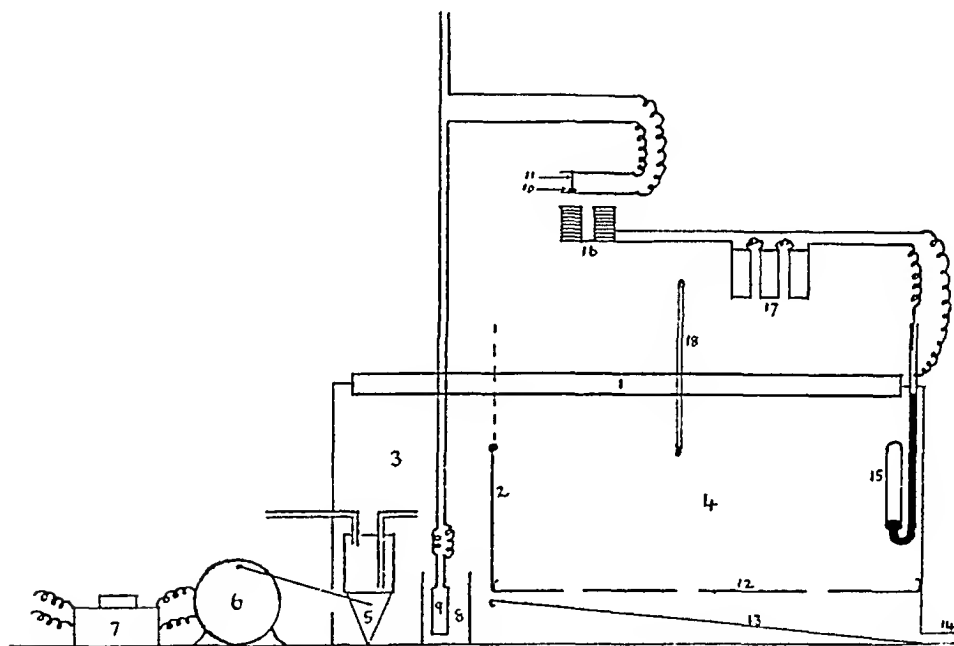


Fig 3

of a shaft passed through a slit in the cabinet, to the eccentric wheel of the shaking machine motor (6). The speed of the rocking is controlled by a rheostat (7).

The smaller chamber (or larger chamber) contains a pot (8) holding water in which there is placed an immersion electric heater (9). The circuit of this heater is closed by the contact of a disc (10) (belonging to an electromagnetic relay), with a platinum pin in the circuit (11).

The floor of the larger chamber consists of a perforated and removable copper pan (12). Beneath this there is a sloping floor (13) which conducts waste fluids through the drain pipe (14).

Through the roof of this chamber there is inserted a toluol-mercury thermoregulator (15) which opens and closes (by means of the mercury columns) a circuit energizing the electromagnet (16). Several dry batteries

(17) or other sources of current, are placed in series through this circuit. A thermometer (18) records the temperature of the large chamber.

By this method a very accurately and automatically controlled temperature is obtained in the large chamber. The immersion heater (9) evaporates the water in the pot (8) and raises the temperature in the cabinet. The excess moisture condenses and the humidity is therefore practically constant for a given temperature. As soon as the temperature reaches the desired level, the mercury columns in (15) close the circuit energizing the electromagnet (16). The latter attracts the disc (10) and pulls it away from contact (11). This in turn breaks the circuit and cuts off the current from the heater (9). The temperature then begins to fall, the mercury column in (15) contracts, the electromagnet circuit is broken, the disc (10) springs back to contact (11), and the heater is immediately put on again. It is advisable to put a drop of cedar oil between contacts (10) and (11) to avoid sparking.

In review, we have three sections: the pressure section automatically delivers gas at constant pressure; the saline section automatically delivers washing fluid at a constant temperature; the injection section keeps the organ to

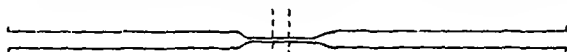


Fig. 4

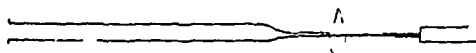


Fig. 5

be injected in a cabinet at constant temperature and humidity. Moreover, the fluid to be injected is agitated by a standardized motion so that if a suspension of particulate matter is used, such as a barium sulphate suspension, a uniform injecting medium is obtained.

TECHNIC OF INJECTION

A typical example of injection to serve as an illustration will suffice to explain the technic employed. If it is desired to inject the coronary arteries of the heart, glass cannulas are inserted into the orifices of the coronary arteries, and are tied into these vessels by means of silk thread. Since the first branches from the coronary arteries are often given off very close to their origin, it is necessary to employ cannulas having a very flat flange. The cannulas are best made in the laboratory and by means of the following technic, they can be made strong enough and at the same time fine enough to be inserted into the coronary arteries of the hearts of newborn infants.

Pieces of glass tubing 3 mm internal diameter, 5 mm external diameter and about 10 cm long are used. The center of the piece of tubing is softened in the flame of a blow pipe, slightly thickened and drawn out (Fig. 4). The drawn out ends are nicked with a file about 1 cm from the slope of the shoulder and broken off.

The nozzle is dipped into talcum powder, a teasing needle is also dipped into talcum powder and its point is inserted into the lumen of the nozzle (Fig. 5).

The tube is slowly rotated around the point of the needle and is gradually introduced into the flame of a blow pipe. It is necessary that the flame be kept small. The tip of the

nozzle immediately begins to thicken and the lumen is kept open by the needle. The purpose of the talem powder is to prevent the needle from fusing with the glass. When the desired thickness of flange is obtained, the tip is removed from the flame, and the opposite end of the tube is rounded in the flame.

The heart is suspended on a tripod, by means of a glass rod which is inserted beneath the pericardial bridge connecting the great vessels, and is placed in the larger chamber. Warm saline (45° C) is forced through the coronary arteries until the washings are free of blood (about fifteen to twenty minutes). The pressure at which the washing is carried on should be about 180 mm Hg.

The pressure tube is now applied to the inlet tube of a Wolff bottle which is mounted on the rocker and which contains the injecting medium¹. If the injecting medium consists of a suspension, such as barium sulphate in gelatin, the rocker is set in motion so as to keep the suspension uniform. The injecting medium is forced through the outflow tube of the Wolff bottle, through the cannulas into the coronary arteries. The pressure is again maintained constant.

When the arteries are completely injected, as can be judged from the fact that after shutting off gas, the mercury column in the manometer does not fall (this takes from twenty-five to thirty minutes), two clamps are placed on each rubber tube connected to the cannulas. The rubber tubing is cut between the clamps. The heart is immediately plunged into cold water, excess barium is washed out of the heart chambers by a stream of cold water, or by means of suction, and the heart is immersed in 10 per cent formalin. After twenty-four hours the heart may be opened and such studies made on it as desired. If it is desired to render the heart transparent, the Spalteholz method or a modification¹ may be used.

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AN OPERATING BOARD FOR RABBITS*

BY EMIL WEISS, M D, CHICAGO, ILL.

SEVERAL apparatuses for the operative work on rabbits have been described and recommended. A good apparatus of this kind should fulfill two conditions. First, the animal should be securely fixed without injury, and secondly, the position of the animal should be such that it will not impede the intended operation. Usually two different positions are required. First, the fixation on the back, and secondly, on the abdomen. For operations on the abdomen, thorax, and neck the dorsal fixation is required, and for operations on the head the abdominal fixation is preferable. The majority of apparatuses are useful for either one or the other position, but not for both.

*From the Department of Bacteriology, Pathology and Preventive Medicine, Loyola University School of Medicine and St. Ann's Hospital.
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We have devised an apparatus, which has some advantages over the present ones in use. The apparatus is attached to a revolving chair, and to the feet of the chair are affixed castors. By this arrangement the apparatus is portable, rotatable in horizontal direction and can be raised at convenience. The stability of the apparatus is assured by placing a twenty five pound weight on the bottom of the chair. The apparatus itself is composed as follows. A horizontal board is screwed to the chair in place of the seat board. To one end of the horizontal board is attached a shorter vertical board, this board is connected on the upper end by hinges with another board, which can

Fig 1

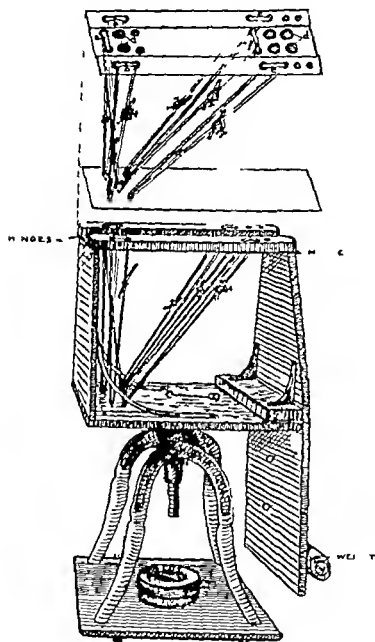


Fig 2

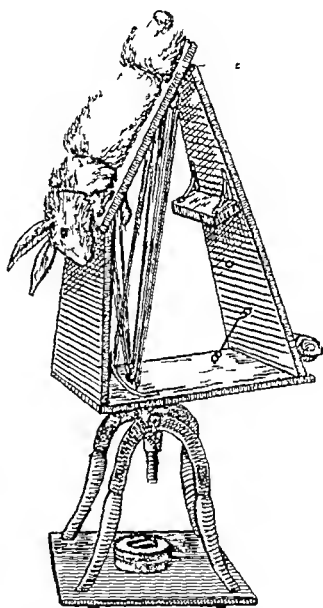


Fig 3

be raised from horizontal to vertical position. The last mentioned board contains larger and smaller holes. The large holes are intended for the feet of the rabbits, the small ones for the rubber tubings used for the fixation of the animal. This board and the horizontal board attached to the chair are of the same length. The perforated board is connected on the other side by hinges with a board twice its length. This board is resting on the lower horizontal board by means of a small piece of wood, which is screwed on to the inner side of the long board, in this position of the long board the horizontal

and the perforated boards be parallel. To the lower end of the long board is attached a weight of five pounds. The long board can be raised in vertical resp. oblique direction, which causes a simultaneous raising of the perforated board from its horizontal position to a more or less oblique position. This particular position of the fixed animal is especially desirable for intraperitoneal injections with head low and abdomen high which causes the fall of intestines toward the diaphragm and avoids injury to the intestines by the intraperitoneal injections. The same position is also desirable for injections in the auricular veins, which become more and more dilated and greatly facilitate these inoculations. To a certain extent heart punctures are more easily made in this position of the animal, due to the fact that the beating of the heart is accentuated.

The animal is fixed to the board, the abdomen downward, by pulling the fore feet through holes *c* and the hind feet through holes *d*. The back of the animal is fixed by a rubber tubing going through holes *f*; the neck of the animal is fixed by a rubber tubing going through holes *a*. A position for abdominal exposure is obtained by pulling the animal, which is lying on its back, through rubber tubing *f*, and fixing the extremities with rubber tubings *b* and *e*. Each tubing from the perforated board goes through the respective eye screw, which is fastened to the lower horizontal board. The ends of the rubber tubings are held fast by a Hoffmann clamp, which affords an adjustment in either shortening or lengthening of the tubing.

Fig. 1 shows the arrangement of the holes and of the tubing on the perforated board and the fixation of the tubing on the lower horizontal board. Fig. 2 shows the entire apparatus and its attachment to the swivel chair. Fig. 3 shows the raised perforated board with a rabbit in a fixed position.

This apparatus insures a satisfactory fixation of rabbits in various positions without any injury; the fixation of the animal can be performed easily by one person. It can also be used for operations of any kind on cats. We have been using this apparatus for several years in the medical school and hospital laboratories.

NOTES ON BASAL METABOLISM*

XI BASAL METABOLISM STANDARDS

BY WILLIAM H. STONER, A.M., M.D., PHILADELPHIA, PA.

INTRODUCTION

THE purposes here are first to present in outline the several standards of comparison of basal metabolic rate in use at present, second to compare the results obtained in the use of these several standards, third to recommend a choice of standards and fourth to evaluate the importance of such a choice. The older aspects of the historical development of calorimetric standards are not presented. Only the present status of the subject is outlined.

The various normals of comparison of basal metabolic rate in use at present are very unsatisfactory. This is true of the standards for adults and those for children are even more unsatisfactory. Several wholly different methods of comparison for adults and for children are in use. Each method gives a different result from the same experimental data. The magnitudes of the variations between these several results are the subject of much difference of opinion expressed in the literature. Each calorimetrist is convinced that the particular set of standards he uses is superior to all the others, yet his knowledge of the advantages and disadvantages of each and of the variations between them is, as a rule, quite uncertain.

STANDARDS

*Aub and DuBois Standards*¹—The oldest of the several systems of standards now in use are those published from the Sage Institute. They consist of a tabulation, for males and females between ages fourteen and eighty years, by two year periods from fourteen to twenty years and by decades from twenty to eighty years of the normal number of calories per hour per square meter body surface based on the DuBois and DuBois height weight formula.²

From the Biochemistry Laboratory and the Department of Metabolic Disease of the Graduate School of Medicine of the University of Pennsylvania. Read before the American Association for the Study of Colter Feb. 1, 1926 at Louisville, Ky.

Received for publication March 15, 1926.

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- V Tables of Values for Dreyer's Formula Boston Med and Surg Jour 1923 clxxxix 39
- VI Complementary Tables of Values of Dreyer's Formulas Boston Med and Surg Jour 1924 cxci 106
- VII Actual vs. Theoretic Weight in Dreyer's Formula Boston Med and Surg Jour 1924 cxci 1030
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- IX Simplified Calculation for Gasometer Gas Analysis Method Jour Lab and Clin Med 1927 xli 884
- X Simplified Data Blank for Gasometer Gas Analysis Method Jour Lab and Clin Med November 1927 xli No. p. 164

Although a smooth curve of these values for males accompanied the original communication, the uninterpolated step curve values have been used generally. The values for females are approximately 7 per cent below those for males.

The most frequent criticism of the Aub and DuBois standards, as they are generally used, is that they are not interpolated between the decades above twenty years, nor between the two-year periods below twenty years. For example, the normal heat production by these standards of a man thirty-nine years old will be the same as that of a man twenty years old and of the same body surface, and 25 per cent more than that of a man forty-one years old and of the same body area. It is interesting to note that much time, energy and money are expended in the elimination of errors of as little as one one-hundredth this magnitude from basal metabolic rate determinations. To overcome these inaccuracies several interpolations, in addition to the original one by Aub and DuBois, have been made.

Some of these interpolations have been made by centering the stated value in the decades and the two-year periods. Others place the stated value at the zero, or one, or beginning of the decade, and still others at the nine, or ten, or end of the decade. These interpolations are made graphically on cross-section paper or by actual mathematical calculation by the usual method of successive differences.

Several variations and extensions of the original Sage standards have been used. Means and Woodwell³ showed that, if from each value in the Sage standards there be subtracted 18 calories, results are obtained which are, on the average, practically identical with those obtained from the Harris and Benedict values. They not only did not recommend so changing the Sage standards but definitely recommended retaining them unchanged. Sanborn,⁴ however, introduced this unrecommended standard into his percentile system of calculation of basal metabolic rate issued with instructions for the use of his respiration apparatus.

The data card accompanying the calorimeter extends the Sage values so as to cover ages six to fourteen years by rather irregular two year steps. Krogh has recently recommended the Sage standards reduced by 6 per cent.

The values of the Sage formula for surface area are given in the graph appearing in the original publication. Tabular arrangement of the values are given in No. VIII of this series of notes.

Harris and Benedict Standards—Harris and Benedict,⁵ considering the surface area law to be at best but an empirical formula, correlated twenty-four hour heat production upon direct physical measurements. They presented formulas, derived statistically from a series of normal subjects, to the values of which the individuals of the series conformed with least error when correlated in relation to sex, weight, stature and age. For convenience in use they presented tabular values of these formulas for individuals between twenty-one and seventy years old. Since the original communication Benedict has stated the formula for males to be applicable to subjects from one year to old age. Females below eighteen years old, however, show considerable variation from the values of the formula for adults.

Dreyer Standards—Dreyer,⁶ analyzing the data used by Harris and Benedict in deriving their formulas, showed that stature is not a legitimate biometric measurement upon which to correlate heat production. He derived statistical formulas from the values of which the variation of individuals of this normal series is less than from the values of the formulas of Harris and Benedict, since the biometric measurement, stature, is omitted. Correlation of heat production was made wholly upon sex, age, and actual weight. Dreyer, in the original preliminary communication, stated that still closer correlation would be obtained by substitution for actual weight, a theoretic normal weight based upon his formulas correlating weight with resting chest circumference and sitting height. The original Dreyer formula is probably more accurate when actual than when theoretic weight is employed, and when theoretic weight is employed, some modification of the formula should be applied.

Hobson,⁷ in Dreyer's laboratory, writes that since "the measurements necessary to determine the 'normal (theoretic) weight' of the individual of Benedict's series were not available and the values published in his (Dreyer's) formula were derived from the observed weight it is possible that these values may require some slight modification." He correlates the daily heat production of 46 normal men with age and theoretic weight based upon sitting heights and chest circumferences and concludes that for individuals leading a healthy active life, Dreyer's formula should be modified by approximately 25 per cent.

It may be concluded, therefore, that theoretic weight based upon trunk length and chest circumference has no advantage over actual weight in predicting heat production by Dreyer's original formula. Tabular values of this formula for males from ages five to eighty have been published.⁸ The values of the formula for females are 10 per cent below the corresponding values for males.

Standards for Children—The present standards of heat production for the calculation of basal metabolic rates of children are even more widely divergent and unsatisfactory than the standards for adults. Probably the most used are those of Auh and DuBois for children not under fourteen years old, of Benedict⁹ for girls twelve to eighteen years old, of Benedict and Talbot¹⁰ for girls under twelve years, and of Harris and Benedict for boys of all ages over one year.

Dreyer and Hobson state that the Dreyer formulas are applicable to all individuals except those under five years old. It is interesting to note that as Dreyer ignored stature in his formulas for adults so did Benedict and Talbot not consider this biometric measurement to be related to heat production of children of certain ages.

VARIATIONS IN RESULTS

Much uncertainty exists in the literature concerning the magnitude of the variations in the results of basal metabolic rate determinations by employing the various standards.

Several series of determinations have been reported in which the basal metabolic rates were calculated, using both the Aub and DuBois standards and the formulas of Harris and Benedict. Among these are the series of Boothby and Sandiford,¹¹ who report 404 comparative tests in which the rates by the Harris and Benedict method are 6.5 points above those by the Aub and DuBois method, 455 comparative calculations substantially confirming this. Means and Woodwell find 6 points deviation in the same direction. In another series⁸ of 350 routine hospital determinations, the Harris and Benedict standards yield results 3 per cent above the Aub and DuBois standards and the Dreyer formulas give rates 0.3 per cent above those of Harris and Benedict.

Very few comparative series have been reported using the Dreyer formulas, probably because of the difficulty of calculation.

CHOICE OF STANDARDS

Three main factors determine the choice of basal metabolism standards—first custom, second, scientific foundation, and third, availability.

The older metabolism clinics adhere conservatively to the standards that have been used since their beginning thus making all their results comparable, while the younger clinics, having few or no comparative data on file, lose nothing by adopting the newer and possibly more scientifically based standards of comparison.

Those to whom the arguments of Harris and Benedict concerning surface area appeal use these standards in preference to those of Aub and DuBois, while those to whom Dreyer's argument against stature appeals employ his formula.

The Aub and DuBois standards are the most available of the three main systems. The table of values in calories and the height-weight graph of surface area have been reproduced in numerous articles and monographs.

The Harris and Benedict tables of values are available in the Carnegie Institution monographs, in Benedict's contribution to Abderhalden's *Handbuch* and in Joslin's¹⁴ textbook. In consequence these standards are used less than those of Aub and DuBois.

The formulas of Dreyer, requiring logarithmic solution, have not been extremely popular among the rank and file of clinicians. Values of the male formula have been tabulated and published in Nos. V and VI of this series of notes, so that these standards need no longer be shunned on account of the mathematical difficulty. In fact, with these tables available, the Dreyer values are the simplest of any to apply to the determination of basal metabolic rate. Each of the others requires the use of two factors selected from tables or graphs while the value of Dreyer's formula is taken directly from a table as a single number. Another advantage of the Dreyer formula is that it is applicable in unchanged form to all ages from five to senility. Only one table of values is required for males and females as the values for females may be derived from those for males by deducting 10 per cent.

IMPORTANCE OF CHOICE OF STANDARD

It is becoming more and more generally accepted that basal metabolic rate determinations are of less value in diagnosis than in following the prog-

ress of treatment—surgical, radiologic, medicinal or hygienic. For this reason the importance of the present unsatisfactory nature of metabolism standards is minimized. Even though by the various standards one does obtain widely variant results from the same experimental data, in following the course of one particular case, if the same standard be used in successive estimations changes in the metabolic rate may be satisfactorily estimated. For the purpose of following the course of treatment of a case it is not necessary to use any standard of comparison. The basal metabolic rate may be expressed, for such individual comparative determination as total calories per hour or day, or in still simpler terms, as the oxygen consumption per minute, hour or day. Certain clinics have recently taken a step in this direction by expressing metabolic rate not as a percentile variation of actual from expected normal heat production, but in actual calories per hour per square meter or in actual total daily calories. This leaves the diagnostic interpretation to the clinician's choice of standard and is perfectly satisfactory in interpreting the results of successive determinations in the study of the progress of an individual case under therapy.

The most important point in the whole subject of basal metabolism standards is for the clinician to know that there are more standards of comparison than for hemoglobin and that just as in the case of hemoglobin studies, it is necessary for him to know the relative values of each in order to make proper diagnostic interpretations.

SUMMARY

The various present day standards of reference for basal metabolic rate determinations of adults and children are outlined and compared. With a view to simplicity and uniformity, the Dreyer standards are recommended for the younger clinics without accumulated experimental data employing the older standards.

The variations between the results obtained from the various standards are less significant since the use of basal metabolic rate determinations has become almost wholly transferred from the field of diagnosis to that of therapeutics.

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WASSERMANN REACTION IN CEREBROSPINAL FLUIDS CONTAINING BLOOD*

By ALVIN G. FOORD, M.D., AND MARJORIE BAUCKUS,
BUFFALO, N. Y.

RECENT literature and many of the standard textbooks of laboratory diagnosis contain references to the effect of blood in cerebrospinal fluid upon the usual tests for globulin¹ and the colloidal gold or other colloidal reactions, but its effect upon the Wassermann test has not been fully covered. Some techniques described advise centrifuging the sample free from blood cells, inactivating the supernatant fluid, and conducting the test upon it. The theoretical objection to such practice is that small amounts of Wassermann-positive blood traumatically introduced by the spinal puncture needle into the cerebrospinal fluid may influence the Wassermann test thereof, since the volume of cerebrospinal fluid used in a Wassermann test is usually ten or more times the volume of serum usually used in standard techniques. Also, sera reported as four plus by most qualitative techniques are frequently much more potent than reported and many times will give four plus reactions when diluted ten times or more and, therefore, are really 40 plus or more (Table I). Consequently the Wassermann test on cerebrospinal fluid containing blood multiplies the effect of the use of large amounts of the fluid by the commonly high potency of the contained blood. This is particularly interesting in those cases in which the blood Wassermann is positive and a cerebrospinal fluid examination is made to determine the presence or absence of central nervous system involvement. In such cases cell counts are questionable when blood is present, even in microscopic amounts, and likewise the globulin and colloidal reactions are vitiated.

Our first experiments consisted of adding various small quantities of pooled Wassermann positive sera to pooled cerebrospinal fluid, negative in all regards (normal cell count, globulin and colloidal gold reaction, and negative Wassermann reaction), and determining the Wassermann reaction of the mixtures by a technique practically identical with that of the New York State Laboratory at Albany from which the antigens and amboceptor were received. The amounts tested with both the plain alcoholic and cholesterolized antigens were 0.4 c.c. and 0.2 c.c., the total volume of the test being 1.2 c.c. The mixtures were inactivated at 56° C. for twenty minutes before testing.

Table I shows that as little as 0.01 c.c. of positive serum in 2 c.c. of normal cerebrospinal fluid imparts enough Wassermann "reagin" to give a slightly positive test, and that as little as 0.04 c.c. will impart a four plus reaction to negative cerebrospinal fluid. Readings in salt solution were occasionally a little stronger.

*From the Laboratories of the Buffalo City Hospital.

Received for publication March 25, 1927.

¹Feinberg, S. M.: Value of Ross-Jones Test on Bloody Spinal Fluid. *JOUR. LAB. AND CLIN. MED.*, 1921, vol. 6, 42.

TABLE I

AMOUNT OF SERUM (CC)	POSITIVE FOOLED SERUM IN 2 CC NEGATIVE SPINAL FLUID										POSITIVE FOOLED SERUM IN 2 CC NORMAL SALT									
	2/2/24		2/12/24		2/28/24		3/7/24		3/14/24		3/22/24		2/-/24		2/12/24		3/14/24		3/22/24	
	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH
0.01	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.02	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
0.03	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.04	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.06	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.08	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.09	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

AL = Plain alcoholic antigen

CH = Cholesterolized antigen.

Next, instead of positive pooled sera, we used single fresh whole blood samples taken at random from forty serologically syphilitic patients, the vast majority with late lues, and added various quantities (from 0.005 cc to 1 cc) to 2 cc of negative cerebrospinal fluid. The amounts of blood added made the spinal fluids grossly bloody, those with the larger amounts decidedly so. Microscopic amounts were not used, because experience taught that such quantities of blood were of no importance. These were then freed of cells, and inactivated at 56° C for twenty minutes before testing. Table II shows the results.

TABLE II

VOLUME SERUM BLOOD ADDED	TOTAL TESTS	ALCOHOLIC ANTIGEN						CHOLESTEROLIZED ANTIGEN					
		-	±	1+	2+	3+	4+	-	±	1+	2+	3+	4+
cc													
0.10	36	12	3	1	6	4	8	0	0	4	6	6	18
0.07	3	1	0	2	0	0	0	0	0	1	1	1	0
0.05	29	15	4	4	1	4	1	4	3	5	7	4	6
0.03	12	9	1	0	2	0	0	4	3	1	1	2	1
0.02	12	9	1	2	0	0	0	7	3	1	1	2	1
0.015	5	5	0	0	0	0	0	1	0	2	2	0	0
0.010	7	6	1	0	0	0	0	2	0	0	3	2	0
0.005	3	3	0	0	0	0	0	2	1	0	0	0	0

The reactions (Table II) were less on the average than when serum was added, which was to be expected, since the serum constitutes roughly about 50 per cent of the blood volume. Partial fixation resulted in five out of seven tests when 0.01 cc of blood was added to 2 cc of negative cerebrospinal fluid, when 0.1 cc was added, complete or nearly complete fixation was noticed in over half of the tests (alcoholic antigen showed 3-plus or 4-plus in twelve out of thirty-six and the cholesterolized antigen in twenty-four out of thirty-six). Many of the patients from whom blood was drawn were under treatment, and if picked patients had been used instead of the ordinary run of hospital patients, the results, no doubt, would have been much more striking.

SUMMARY AND CONCLUSIONS

It has been demonstrated above that only very small amounts of Wassermann-positive blood need be introduced into normal cerebrospinal fluid to impart to the latter a positive Wassermann reaction, this being due to the fact that the test conducted with cerebrospinal fluid is done with relatively large amounts of fluid (ten times the usual serum amounts in most techniques), and also because the usual four plus Wassermann sera are usually much stronger than four plus.

In examining cerebrospinal fluids from patients showing a positive blood Wassermann we strongly advise against the use of fluids containing traumatic blood in grossly visible amounts. Negative reactions are as reliable as in those not containing blood, but a positive reaction may be due either to neurosyphilitic disease or to the contained blood.

FROZEN SECTIONS THEIR VALUE AS A ROUTINE PROCEDURE*

By ROBERT A. KELTY, M.D. WASHINGTON, D. C.

THE purpose of this paper is to present some of the facts in favor of frozen tissue sections as a routine procedure. Having had several inquiries lately as to the method in use its importance might deserve a paper.

Frozen sections may be arbitrarily divided into two groups. First, those sections which are removed by biopsy for immediate diagnosis, and second, those sections which are fixed for a variable period and cut by the frozen method. The latter includes the routine surgical and autopsy sections.

The sectioning of fresh tissue removed by biopsy for immediate diagnosis has its field, and the advantage of this chance for diagnosis might be used more often than it is. I think one of the reasons for its lack of universal popularity is its infrequent use. Nobody about the laboratory in practice, the apparatus is frequently out of order the stains are not any too good and, of course, the finished sections show nothing but a poorly stained field full of artefacts. If for no other reason practice in making frozen sections and apparatus in perfect order would be worth the effort to get good sections for biopsy diagnosis. In my own work I use exactly the same technique for fresh frozen sections as I do for the routine staining with hematoxylin eosin and not with polychrome methylene blue. There is no objection to the latter and where as many slides as are made in MacCarty's laboratory at the Mayo Clinic are examined by this stain I am sure one would like the picture quite as well as one made with hematoxylin eosin. I prefer the latter stain, and while it makes the whole process a little longer—possibly five minutes—one obtains a good well cleared stain, and, being familiar with the stain, the diagnosis is much easier to make. The slides are permanent.

For routine work the fresh surgical specimen is fixed in 4 per cent formalin, i.e., 4 cc of 40 per cent formalin in 100 cc of distilled water. The whole specimen or a small section may be fixed. It should have plenty of fluid in a bottle large enough to hold it. Lightning top preserve jars will be found quite convenient for most specimens. Special care is necessary in putting up specimens removed by enucleation. These are picked and placed together in a little pile on a single layer of gauze and the loose ends of the gauze are held together with a small elastic band. The bill is made just tight enough to hold together. This is placed in the fixing fluid and the whole after fixation is frozen and cut right through the gauze. The section when floated on water separates into the component parts of the scrapings,

* From the Laboratories of the Diagnostic Centre U. S. Veterans Bureau Washington, D. C.

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and care is taken to have all types of small pieces mounted. This little trick overcomes about the only difficulty met with in freezing and cutting as far as the type of tissue is concerned.

Tissues are fixed in the formalin for twenty-four hours, very occasionally they may require a longer period but not often. At the end of this time they are frozen with carbon dioxide and cut. In freezing, a little practice determines just the proper time for cutting which usually lasts from about thirty seconds to a minute when all the sections necessary may be cut. Sections may be cut thin, but eight to ten microns will give good sections in most cases. One of the secrets of good sections is a sharp knife. Several knives should be kept on hand in perfect condition. It has been found that it is better to sharpen them immediately after using and put them away ready for the next time. The type of stone or strop used depends on the individual operator. I prefer a very fine oil stone followed by plenty of stropping. The Spencer automatic freezing microtome is very satisfactory.

With a little water the sections are floated from the knife into a small container of lukewarm water. The container should be black since it aids as a background to the white sections. All manipulation of straightening is done at this time in the water and none after it is mounted on the slide. For straightening in the water a small glass rod, a fine brush, or a very sharp pointed steel needle may be used. After the section is spread out it is quickly mounted on the glass slide. If it is not smooth on the slide put it back in the water or take another section. Any straightening on the slide produces artefacts. The water is drained off. If the section is fresh frozen biopsy material, the slide is now flooded with 10 per cent formalin for one minute followed by 95 per cent and absolute alcohol, blotting after each fluid. If the tissue has been fixed the formalin is omitted. The alcohol is drained off, and the section is blotted with filter paper tight against the slide. It will spoil at this time if it is allowed to dry more than a few seconds. The section is then covered with three or four drops of thin celloidin in equal parts of absolute alcohol and ether, and with one blow the celloidin is evenly spread over the section. A little practice will make this perfect. If the celloidin is too thick it will diffuse the stain and mask the specimen, if it is too thin the section will come off in the stains, it is not hard to get it just right. The section is then ready to stain.

- 1 Stain, Delafield's hematoxylin, this should be old, ripe, and good and thick, and not frequently filtered. A good stain takes about two minutes. Use Coplin jars for all solutions.
- 2 Wash off excess in water.
- 3 Take out further excess in the section until light brown in 2 per cent hydrochloric acid alcohol, after fifteen seconds.
- 4 Develop blue color in ammonia water, about fifteen seconds. A few drops of pure ammonia water in a Coplin jar of distilled water.
- 5 Wash in water to clear ammonia.
- 6 Dehydrate with 95 per cent and absolute alcohol, a few seconds each.
- 7 Stain in saturated alcoholic eosin, if stain is right, about thirty seconds.
- 8 Clear out excess in two jars of 95 per cent alcohol and one of absolute.
- 9 Clear in carbol-xylol.
- 10 Clear out in two jars of plain xylol.
- 11 Mount in balsam.

With a little practice excellent sections can be obtained quickly. I have not cut a routine surgical or autopsy pathologic section in over four years by any method other than by frozen section, and this answers the question as to what type of tissue lends itself to this method. The answer is, all tissues.

Carbon dioxide tanks may be easily procured. It takes about four tanks, one in use, one in transit, one in stock and one for emergency. The tank in use should be placed horizontally above the freezing machine, the outlet end should be about two inches lower than the base. This obviates tank troubles. The ordinary tank cuts on an average of thirty five specimens and costs about four dollars. Carbon dioxide may be used extensively for commercial ice packing in the near future. This will lessen this cost decidedly.

The big advantage in frozen sections is the rapidity with which they can be done, the fact that sections can be cut, examined, filed and reported out of the way at least the day following an operation. It gets the work out of the pathologist's hands promptly and into the surgeon's hands while the case is still fresh in his memory. As an example in autopsy work I recently had an autopsy at noon and presented the finished case with projection of the microscopic slides at the staff meeting the same evening. This was a decided advantage both to me and to the staff.

Frozen sections have no real disadvantage. There are many common objections, such as that the sections are too thick, the picture is incorrect, the stain is poor, or there are artefacts. None of these are real. The sections may be thicker than the two micron paraffin sections, but instead of being an objection this is often an advantage. One can see the whole cell and more especially one can get the true relationship of one cell to another without the parboiled appearance of the paraffin. The picture instead of being incorrect is in reality the true picture of the individual characters of cell protoplasm and not the fixed hard picture of paraffin. By the method outlined the staining contrast of the hematoxylin eosin is quite beautiful. Artefacts can be entirely eliminated by practice and in only the delicate lacy textures of some structures like the thyroid will they be present. This can even be eliminated by a little longer primary formalin fixation.

SUMMARY OF METHOD FOR ROUTINE FIXED FROZEN SECTIONS

- 1 Fix small pieces of fresh tissue twenty four hours in formalin
- 2 Freeze and cut 8-10 microns
- 3 Float sections onto warm water in dark container
- 4 All manipulations should be delicately made in the water
- 5 Mount section straightened out on a microscopic slide
- 6 Drain off excess of water and blot with slight pressure
- 7 Dehydrate 95 per cent absolute alcohol and blot dry
- 8 Cover by one blow with a thin layer of thin celloidin
- 9 Stain with hematoxylin two minutes
- 10 Wash off excess of stain in water
- 11 Further excess of stain removed from section in acid alcohol, 15 seconds
- 12 Develop blue color in very weak ammonia water 15 seconds
- 13 Wash out ammonia in water
- 14 Dehydrate 95 per cent and absolute alcohol few seconds

- 15 Stain with saturated alcoholic eosin, 30 seconds to one minute
- 16 Wash off excess of eosin in three changes of alcohol
- 17 Clear in carbol xylol
- 18 Clear in two changes of plain xylol
- 19 Mount in balsam

Frozen sections are worth a try, and once one has seen their advantages one is wedded to them for routine purposes

A MODIFICATION OF THE ORSKOV SINGLE CELL CULTURE TECHNIC*

By ESTHER WAGNER STARN, PH D, AND ALLEN E STARN, PH D,
COLUMBIA, Mo

INTRODUCTION

IN 1922 Orskov¹ published a method for obtaining single cell cultures which is capable of rather easy application as compared with those previously described. Several changes in his technique, developed by us during the course of a series of investigations on bacterial variation, have led to a modified technique which is capable of results as clean-cut, and as reliable, as those obtained by using Orskov's original method, and yet which eliminates even more of the tediousness of the older methods than does his, giving a simple method, easy of execution, and yielding practically unquestionable results.

OUTLINE OF METHOD

1 *Obtaining a Single Cell*—Slides, marked as described below, are placed on a piece of filter paper in a Petri dish and sterilized in the oven in the usual manner. Clear centrifuged agar media is then allowed to flow on the slide from a sterile capillary pipette. For the successful preparation of a level agar surface four conditions must be fulfilled:

1 A high dilution of nutrient agar should be used in place of the usual concentration of agar. Diluted sugar agars may be used to advantage at times.

2 The agar solution, when poured, must be clear. It is therefore filtered and centrifuged.

3 A capillary pipette should be used to obtain a smooth flow and to give a thin layer of agar.

4 The glass slide should be hot during the pouring in order that the agar shall spread evenly in place of setting locally in lumps. Usually the pouring is done just after removing the slide from the sterilizing oven.

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¹Orskov, J. Method for the Isolation of Bacteria in Pure Culture from Single Cells and Procedure for the Direct Tracing of Bacterial Growth on a Solid Medium. Jour. Bacteriol. 1922 vol. 537

A series of these plates is prepared at one time and placed in the refrigerator for from two to twenty four hours for hardening. To insure the sterility of the agar, the plates are incubated for twenty four hours and then examined.

A two hour bacterial culture, which is much to be preferred to a twelve hour one, is then used for streaking. The streaking is done with a very delicate platinum wire, which has been found to make the clearest lines. Streaking is preferred to spreading because of the increase in definiteness of the exact location of the organism. It has the added advantage that, as the colony under observation develops there are only two directions to watch for the overlapping of other colonies from adjacent cells, in place of a large number. After streaking the plates are incubated for from one half to one hour, then examined, and then again incubated.

2 *Location of Isolated Organism and Relocation of Selected Organism on Colony*—Diagrams, of the design shown by the continuous lines in Fig 1, are scratched, using diamond on glass slides of good quality. After trying many different designs, this simple one was found to be best adapted and most adaptable to this method. The shifting of objectives, etc. often caused confusion when a more complex design was used.

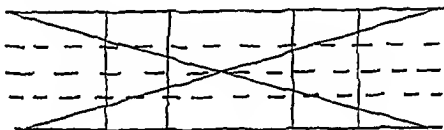


Fig 1

We have not found it necessary to use a micrometer scale attached to the eyepiece as Orskov advises. The streaks represented by the broken lines in Fig 1, take the place of such a micrometer scale and make the 'line pattern' for locating any particular colony a matter of certainty.

By means of the scales of the mechanical stage one can readily find the approximate position of a field on the diagrammed slide. The low power objective is used to obtain the exact position by focussing on a certain angle produced by the lines of the diagram, i.e., the pattern made both by the streaks and the lines scratched on the slide, and this 'line pattern' can then be noted. An exact drawing of the diagram of the slide and streaks is made even before placing on the microscope stage.

The high power objective is used to locate the presence of isolated organisms along any streak. These are marked on the drawing and thus several single organisms can be definitely noted on the same slide.

After finding and marking the positions of the isolated organisms the slide is removed from the stage the ends passed swiftly through a flame and it is then returned to the Petri dish and incubated. After incubation for varying periods of time the positions of the isolated colonies can be compared with those of the originally isolated cells. The filter paper in the Petri dish in which the slides are placed for incubation should be kept slightly moist.

3 *Subculturing from the Single Cell Colony*—With plates which have been properly streaked it is always possible to locate colonies at a sufficient distance from adjacent ones so that transfers to tubes can be made directly from the marked colony in the ordinary manner. It is then merely necessary to note, under the low power of the microscope, the colonies next adjacent on either side to make sure that they have not been disturbed.

DISCUSSION

The above-mentioned modifications of Orskov's original method were adopted, not due to greater reliability of results obtained by their use, but because of the more readily acquired facility of manipulation and a smaller expenditure of time.

The principal differences between the modified and the original Orskov method are outlined below.

1 By use of centrifuged, very soft agar, poured by means of a capillary pipette directly on a sterile *hot* slide, thin, even layers of agar are obtained, thereby meeting Orskov's objection to the use of this method, and at the same time eliminating the necessity for his technique of transferring an already hardened agar slab from a Petri dish. The authors have found that this latter procedure is difficult because of the tendency of strips of agar which are sufficiently thin for the best results to curl and fold. When strips thick enough to be easily transferred in this manner are used, the images are blurred.

2 The use of the diagram shown in Fig. 1 is much simpler both for locating a precise area and for mapping a complete slide with respect to the location of the suitable single cells, than the more complicated patterns used by Orskov.

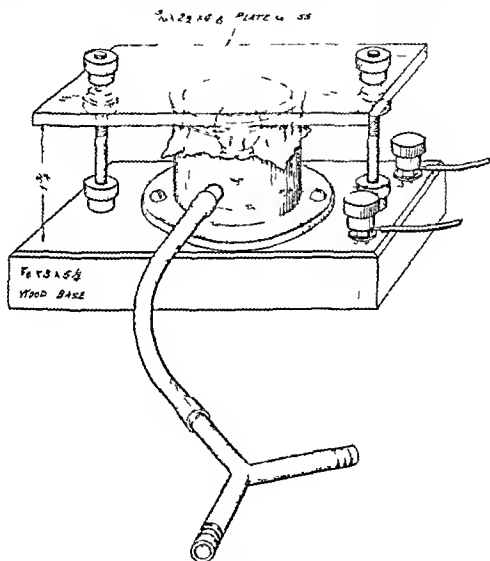
3 Streaking, rather than spreading, the inoculum overcomes certain disadvantages. Orskov states that some workers will prefer to streak, but he does not point out in any way its possible advantages, nor does he seem to consider it in any light other than as a possible alternative, with little to give either it or spreading any preference. In the first place streaking eliminates the necessity for the micrometer in the eyepiece, the "line pattern" being obtained by the superposition of the streaks on the pattern scratched on the slide. In the second place it reduces enormously the number of directions one has to watch for contaminating growth during the development of the single cell colony. One can always locate the streak, and it is a very simple matter to follow it until the organisms become sufficiently far apart so that, when they develop into colonies, transplants can be made from one of these without resorting to the somewhat tedious microscopic technique given by Orskov.

SIMPLE APPARATUS FOR REPEATED BLOOD PRESSURE DETERMINATIONS IN RABBITS*

By THEODORE L. SQUIER, M.D. MILWAUKEE, WIS.

WHEN repeated blood pressure determinations in rabbits are required, a simple nonsurgical method of making the determination is of great assistance. The apparatus I have used is a modification of that described by H. C. Anderson¹ and of that described by T. Kuraya.²

A brass cup 2 inches in diameter is mounted, as shown in the accompanying diagram, on a wooden base measuring 3 by 5½ inches. The edge of



the cup is flanged, and a series of shallow grooves are cut around the upper rim to permit a rubber dental dam to be stretched loosely and tied in an air tight manner over the cup. Inside the cup is a flash light socket and lamp bulb with connection through the hose block to binding posts at one end of the block as illustrated. A metal tube screwed into the wall of the brass cup provides connection with a short piece of rubber tubing which in turn, through

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the Y-connection shown in the diagram, leads through one arm to an ordinary mercury manometer and through the other to a hand bulb for applying an pressure

Threaded metal posts at each end of the block support a plate-glass plate $2\frac{1}{2}$ by 5 inches with holes drilled at each end which permit the plate to slip over the supporting posts. The distance between the lower surface of this plate and the upper surface of the rubber membrane covering the cup can be adjusted easily by means of the supporting thumb screws beneath the plate, and after adjustment the plate is held firmly in position by the thumb screws above

TABLE I
ILLUSTRATIVE BLOOD PRESSURE READINGS OF NORMAL RABBITS

RABBIT NO 36			
Dec 18, 25	64 68 66 66 68	Aver	66 mm
Dec 21, 25	62 72 74 70 74	Aver	70 mm
Dec 22, 25	56 60 56 58 56	Aver	57 mm
Dec 24, 25	52 54 54 54 54	Aver	54 mm
Dec 28, 25	60 60 60 58 58	Aver	59 mm
Dec 29, 25	60 58 60 62 58	Aver	59 mm
Dec 30, 25	60 68 66 66 66	Aver	65 mm
Dec 31, 25	64 64 63 62 66	Aver	64 mm
Jan 4, 26	62 58 60 62 60	Aver	60 mm
Feb 17, 26	59 62 63 59 56	Aver	60 mm
RABBIT NO 65			
Mar 13, 26	54 56 54 53 51	Aver	54 mm
Mar 16, 26	57 56 58 62 58	Aver	55 mm
Mar 18, 26	53 53 56 52 50	Aver	51 mm
Mar 22, 26	58 59 59 57 58	Aver	58 mm
Mar 24, 26	56 58 56 60 58	Aver	57 mm
Mar 26, 26	58 63 62 60 60	Aver	61 mm
Mar 29, 26	66 65 64 60 65	Aver	64 mm
Apr 3, 26	53 54 58 54 54	Aver	55 mm
Apr 6, 26	66 65 64 63 63	Aver	64 mm
Apr 7, 26	56 56 56 56 58	Aver	56 mm
RABBIT NO 172			
Apr 12, 26	66 66 66 65 68	Aver	66 mm
Apr 14, 26	66 66 68 66 65	Aver	66 mm
Apr 17, 26	60 65 61 60 62	Aver	62 mm
Apr 19, 26	63 64 62 60 60	Aver	62 mm
Apr 23, 26	60 60 64 64 58	Aver	61 mm
Apr 26, 26	63 64 66 66 66	Aver	65 mm
Apr 28, 26	63 61 65 61 62	Aver	62 mm
Apr 30, 26	68 70 68 70 70	Aver	69 mm
May 3, 26	67 69 69 65 69	Aver	68 mm
May 5, 26	67 66 70 66 70	Aver	68 mm

Blood pressure readings are made in the central artery of the rabbit's ear, the right usually being used because of greater convenience in handling. The rabbit is prepared by shaving the hair from the central part of the ear chosen. The glass plate is adjusted so that it will just clear the ear surface without exerting pressure. The rabbit is held in position with the left hand, and the upper screws are adjusted so that the glass plate is held firmly. Thus, the right hand is free to manipulate the manometer bulb. The pressure within the ear is gently increased until the blood flow in the central artery is just stopped. This is most easily observed through a simple tripod lens resting on

the glass plate. The mercury level in the manometer is then recorded. As a routine I take five readings and record the average of these as the pressure.

The following simple precautions should be observed:

1. The rabbit should be handled gently and care taken to prevent fright or excitement.

2. Approximately the same position of the central artery should be used in making each subsequent reading.

3. The temperature at which the observations are made should be fairly constant.

It is to be recognized that the pressure reading so obtained is considerably lower than that obtained in the carotid by the surgical method.

In Table I are shown examples of blood pressures obtained as described. The apparatus has now been used for about two years and the method seems simple and accurate for purposes of comparative blood pressure study in rabbits over an indefinite period of time.

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STUDIES IN LOCAL ANESTHESIA. VII

THE TOXICITY OF SOME DERIVATIVES OF PARA-AMINO BENZOIC ACID*

BY JACOB SACKS, PH.D., CHICAGO, ILL.

THE experiments reported here were carried out to determine the toxicity of a series of derivatives of para-amino benzoic acid. The compounds were prepared by Dr. Roger Adams of the Department of Chemistry of the University of Illinois. The anesthetic value of these drugs on the rabbit's eye has been reported by S. J. Cohen.¹

Following are the names and formulas of the compounds in this series:

The method used to determine the toxicity was the intraperitoneal injection of the drugs into albino rats. The rats were weighed and the desired volume of a freshly prepared 1 per cent solution of the drug in 0.85 per cent sodium chloride solution injected into the peritoneal cavity of the rat. The animal immediately released, and the time of injection noted. The symptoms observed were essentially those reported by McGuigan and Brough on an other series of these derivatives, except in the case of those derivatives containing a cyclohexane ring (Nos. 22 to 28). With this exception, the following order of symptoms was noted: there was a period of hyperirritability,

*From Laboratory of Pharmacology and Therapeutics, University of Illinois College of Medicine.

TABLE I

NAME	FORMULA	MOL WT
1 A β Diethylamino isopropyl para amino benzoate phosphate	$(p)NH_2C_6H_4CO_2CH(CH_3)CH_2N(C_2H_5)_2H_3PO_4$	348
1 β Diethylamino isopropyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH(CH_3)CH_2N(C_2H_5)_2HCl$	286
2 β Diethylamino n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_2H_5)_2HCl$	286
3 β Diethylamino n heptyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2CH_2CH_2CH_2N(C_2H_5)_2HCl$	342
4 ϵ Diethylamino n amyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2CH_2CH_2CH_2N(C_2H_5)_2HCl$	314
5 δ Diethylamino n butyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2CH_2N(C_2H_5)_2HCl$	300
6 β Di n butyl amino n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_2H_5)_2HCl$	342
7 γ (3 carbomethoxy piperidyl) n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(CH_2CH_2CH_2CO_2CH_3)_2HCl$	356
8 γ Piperidyl n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(CH_2CH_2CH_2)_2HCl$	298
9 γ Di iso amylamino propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_4H_9)_{(1iso)}_2HCl$	370

TABLE I—CONT'D

NAME	FORMULA	MOL. WT.
10 γ Di n amylamino propyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2CH_2CH_2N(C_4H_9)_2 \cdot HCl$ $C_{24}H_{45}O_2N_2 \cdot HCl$	370
11 γ Di allylamino propyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2CH_2N(CH_2CH=CH_2)_2 \cdot HCl$ $C_{16}H_{25}O_2N_2 \cdot HCl$	310
12 γ Allyl n butylamino propyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2CH_2N(CH_2CH=CH_2)(CH_2CH_2CH_2CH_3) \cdot HCl$ $C_{20}H_{35}O_2N_2 \cdot HCl$	326
13 γ Di sec butylamino propyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2CH_2N(CH_2CH(CH_3)_2)_2 \cdot HCl$ $C_{24}H_{45}O_2N_2 \cdot HCl$	362
14 γ Dimethylamino propyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2CH_2N(CH_3)_2 \cdot HCl$ $C_{14}H_{25}O_2N_2 \cdot HCl$	258
15 β Piperidyl ethyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2N(CH_2CH_2CH_2CH_2)_2 \cdot HCl$ $C_{18}H_{27}O_2N_2 \cdot HCl$	284
16 β (3 carbomethoxy piperidyl) ethyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2N(CH_2CH_2CH_2CH_2CO_2CH_3)_2 \cdot HCl$ $C_{28}H_{45}O_5N_2 \cdot HCl$	342
17 β Di iso amylamino ethyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2N(C_4H_9)_2 \cdot HCl$ $C_{24}H_{45}O_2N_2 \cdot HCl$	356
18 β Di n butylamino ethyl para amino benzoate hydrochloride	$(p)NH_2CHCO_2CH_2CH_2N(C_4H_9)_2 \cdot HCl$ $C_{24}H_{45}O_2N_2 \cdot HCl$	328

TABLE I—CONT'D

NAME	FORMULA	MOI WT
19 β Di sec butylamino ethyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(sec))_2 \cdot HCl$	328
20 β Di n amylamino ethyl para amino benzoate hydrochloride	$C_{11}H_{23}O_2N \cdot HCl$ $(p)NH_2C_6H_4CO_2CH_2CH_2N(C_{11}H_{23}(n))_2 \cdot HCl$ $C_{13}H_{27}O_2N_2 \cdot HCl$	356
21 β Allyl n butylamino ethyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2N \begin{array}{c} \\ H \\ \\ CH-CH=CH_2 \end{array} \begin{array}{c} \\ Cl \\ \\ CH_2CH_2CH_2CH_2CH_3 \end{array}$	312
22 4 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$C_{12}H_{21}O_2N_2 \cdot HCl$ $(p)NH_2C_6H_4CO_2CH_2 \begin{array}{c} \diagup CH_2-CH \\ \diagdown CH_2-CH \end{array} > CH N(CH_2)_2 \cdot HCl^*$ $C_{13}H_{23}O_2N \cdot HCl$	298
23 4 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2 \begin{array}{c} \diagup CH_2-CH \\ \diagdown CH_2-CH \end{array} > CH N(CH_2)_2 \cdot HCl^*$ $C_{11}H_{21}O_2N_2 \cdot HCl$	298
24 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2 \begin{array}{c} \diagup CH-CH_2 \\ \diagdown CH_2-CH \end{array} > CH_2 \begin{array}{c} \diagup N(CH_2)_2 \cdot HCl \\ \diagdown CH_2-CH \end{array}$	298
26 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$C_{11}H_{21}O_2N_2 \cdot HCl$ $(p)NH_2C_6H_4CO_2CH_2 \begin{array}{c} \diagup CH_2-CH_2 \\ \diagdown CH-CH \end{array} > CH \begin{array}{c} \diagup N(CH_2)_2 \cdot HCl \\ \diagdown CH_2-CH \end{array}$	298
28 2 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$C_{17}H_{29}O_2N_2 \cdot HCl$ $(p)NH_2C_6H_4CO_2CH_2 \begin{array}{c} \diagup CH_2-CH \\ \diagdown CH-CH \end{array} \begin{array}{c} \\ N(C_2H_5)_2 \cdot HCl \\ \\ CH_2-CH \end{array} > CH_2 \begin{array}{c} \diagup CH_2 \\ \diagdown CH-CH_2 \end{array}$	326
29 2 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$C_{17}H_{29}O_2N_2 \cdot HCl$ $(p)NH_2C_6H_4CO_2CH_2 \begin{array}{c} \diagup CH-CH_2 \\ \diagdown CH-CH_2 \end{array} \begin{array}{c} \\ N(C_2H_5)_2 \cdot HCl \\ \\ CH_2-CH \end{array} > CH_2 \begin{array}{c} \diagup CH_2 \\ \diagdown CH-CH_2 \end{array}$	326

*Stereoisomeric forms No 22 has a melting point of 220°-222° C while No 23 melts at from 263°-264° C

TABLE II

NUMBER OF COMPOUND	SMALLEST DOSE FOR CONVULSIONS MG PER 100	AV TIME TO ON SET OF CONVULSIONS AT DOSE STATED		M L D NO PER KO	AVERAGE TIME TO DEATH		NO RATS USED	NO DEATHS
		MIN	SEC		MIN	SEC		
1A	300	10	50	325	7	20	4	4
1	150	9	00	275	8	35	6	3
2	250	12	45	275	6	50	4	3
3	75	4	15	125	6	05	2	2
4	75	7	05	75	11	50	5	5
5	50	6	50	75	13	35	6	5
6	75	3	15	100	5	25	2	2
7	350	11	30	400	21	40	2	1
8	15	6	05	20	8	35	5	3
9	75	2	00	75	6	15	2	2
10	50	5	35	75	6	55	3	2
11	100	3	40	100	6	00	5	3
12	75	4	40	100	5	25	6	6
13	75	4	50	75	7	55	2	2
14	400	6	50	400	13	05	3	3
15	150	3	50	175	7	20	4	4
16	250	3	10	275	4	00	4	4
17	75	4	40	100	8	30	2	2
18	75	3	55	75	9	20	0	5
19	100	2	35	150	6	35	5	3
20	100	7	15	125	7	40	2	2
21	75	7	50	90	4	20	5	3
22	75	9	25	100	15	05	6	4
23	50	5	25	75	6	45	4	4
24	50	3	45	75	8	25	3	3
25	50	2	50	75	6	15	1	1
26	100	3	05	150	8	05	4	4
Tutocaine	100	8	45	125	6	15	1	1
Cocaine	50	6	05	50	9	00	4	2

during which a touch on the tail would cause a marked jump. Following this there was a paralysis of the extremities, the respiration then became labored, the head was slowly drawn backward and the animal suddenly went into a clonic spasm. This passed off to be followed by another spasm after a short interval. During the convulsions the animal was generally in opisthotonus. Between convulsions, there was usually a relaxation.

After a series of convulsions there would follow a period of coma, then death, by respiratory paralysis. The heart generally continued to beat for about a minute after respiration stopped.

Animals which did not die within twenty minutes were generally fully recovered within an hour after the injection.

The symptoms evoked when the cyclohexane derivatives were used were somewhat different. After the period of hyperirritability, there followed posterior paralysis but without loss of equilibrium. The animal would then suddenly go into a convulsion during which it was in the "Kangaroo" position, i.e., the fore legs were elevated and flexed and the body supported by the hind legs. Equilibrium was generally lost after the first spasm. Death in these cases was also by respiratory paralysis.

Unlike the effects of sublethal doses of the other members of the series the effect of these derivatives did not wear off for several hours. There was a marked hyperirritability for as long as six hours.

TABLE III
COCAINE COEFFICIENTS

NUMBER OF COMPOUND	ANESTHETIC VALUE	TOXITY
1A	0.19	0.15
1	0.67	0.18
2	0.86	0.18
3	0.74*	0.40
4	0.86	0.67
5,	0.71	0.67
6	1.07*	0.50
7	0.00	0.125
8	0.67	2.50
9	1.67†	0.67
10	2.12†	0.67
11	0.67	0.57
12	0.67	0.50
13	1.16*	0.67
14	0.00	0.125
15	0.24	0.29
16	0.00	0.18
17	2.38†	0.50
18	0.69	0.67
19	0.79*	0.33
20	2.38†	0.40
21	0.88	0.55
22	1.02	0.50
23	1.04	0.67
24	0.52	0.67
26	1.00*	0.67
28	0.50	0.33
Tutocaine	----	0.40

*Irritant †Corrosive
Anesthetic value from data by Cohen¹

Table II gives the smallest dose causing convulsions, the average time to the onset of convulsions with this dose, the minimum lethal dose, the average time to the death of the animal with dose, the number of rats used and the number of deaths at this dose. Cocaine and tutocaine are included for comparison. The toxicity of procaine as determined by this method is reported by McGugan and Brough.²

Table III gives the efficiency of the drugs in producing anesthesia of the rabbit's eye, with the duration of anesthesia with 1 per cent cocaine as unity, and the toxicity compared to cocaine as unity.

DISCUSSION

In those compounds of the series in which the aminoalcohol portion of the molecule is purely aliphatic (Nos. 1 to 6, 9 to 14, and 17 to 21), the minimum lethal dose is 75 to 100 mg per kg of body weight in eleven of the seventeen compounds. Some of the corrosive ones, those with 4- and 5-carbon alkyls, have no greater toxicity than those which produce good anesthesia.

Of the remaining six purely aliphatic derivatives, two of high molecular weight, Nos. 3 and 19, have a somewhat lower toxicity. No. 14, which is of low molecular weight and has no anesthetic value, has a correspondingly low toxicity.

Compounds Nos. 1 and 2 show a high anesthetic value combined with a remarkably low toxicity. No. 1 is an isopropyl derivative and No. 2 a normal propyl derivative with the diethyl-amino group in the β -position.

Those compounds containing a piperidine ring (Nos 7, 8, 15 and 16) show marked concordance between anesthetic value and toxicity. No 8, which is an effective anesthetic, is very toxic, No 15 is less effective and less toxic, and the other two have no anesthetic value and very low toxicity.

The cyclohexane derivatives (Nos 22 to 28) show better anesthetic powers than the purely aliphatic ones, but their toxicity is of the same order.

REFERENCES

¹Cohen, S. J. *JOU. LAB. CLIN. MED.* 1927, xii, 983

²McGowan, H. A., and Brough, G. A. *JOU. LAB. CLIN. MED.* 1925, ii, 479

A PRACTICAL TECHNIC IN THE PREPARATION OF SMEARS FOR THE EXAMINATION OF TUBERCLE BACILLI*

BY L. T. BLACK, M.D., DENVER, COLO.

THE old technic used in the examination of smears for the tubercle bacilli and in making comparisons of different smears according to the Gaffky scale is not only of no value for clinical purposes, but very often has a detrimental psychologic effect on the patient. A patient feels that, if the reading of his Gaffky scale is 7 per field today and was only 4 per field four weeks previously, he is clinically worse.

What are the mistakes made according to the old method? We hunt for lentil like particles in the sputum and smear it thickly on the slide. Sometimes we find tubercle bacilli in those particles and sometimes we do not, and it very frequently happens that our laboratory diagnosis is "too many to count" in a case which, from a clinical and x ray standpoint, must be considered as greatly improved. On the other hand we find fewer bacilli in the smear of a patient who, from the clinical standpoint, is definitely worse. In other words, we fool not only the patient but also ourselves.

During the past two years we have been using a technic suggested by Dr Felix Baum, our Medical Director. Dr Baum had used this method for many years in Europe and had had very satisfactory results. The technic is very simple.

The sputum is placed in a bottle or container and whipped for one minute with a wooden applicator around the end of which a piece of cotton has been wrapped. The tubercle bacilli present in the sputum will become adherent to the cotton. The cotton wrapped end of the applicator is then drawn once very lightly and quickly across the slide, leaving a thin smear in which the tubercle bacilli are distributed homogeneously.

This method has been used in hundreds of cases and we have concluded that in many of the cases where we could not find tubercle bacilli according to the old method of smearing the sputum was positive with this technic. The Gaffky scale applied to this technic is obviously of greater value in the determination of the degree of the tuberculous process.

*From the Medical Department, National Jewish Hospital, Denver, Colo.
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CORRESPONDENCE

Sir—The report which I am about to make may possibly be interpreted as an attempt to discredit the work of one of your contributors. Please be assured that such is not the case, and that the only attempt made is to establish the scientific truth of the case, that factor that sometimes so easily eludes the best of us.

In an article entitled "A Study of the Antigen used in the Wassermann Test for Syphilis" in your issue of June, 1926, Vol. 9, page 864, Mr. S. L. Leiboff, Ph.D., of New York in a consideration of the rôle of the antigen in the fixation of complement and in the formation of a precipitate, makes the following statement:

"Heating of the beef heart tissue or of the alcoholic extracts used as antigens in the Wassermann test for syphilis does not affect their complement fixation power but does destroy their precipitate forming property."

On reading this article with interest, and having available a heart extracted antigen which I had prepared several months ago for use in the Kahn test, but which to date I had not used, I determined to check the above quoted statement in so far as it bears on the precipitate forming property of an antigen for the Kahn test. With the correctness of the first part of the statement I was already familiar, having previously successfully used a heart extracted antigen in the Wassermann test.

A known positive syphilitic serum was obtained which had been reported four plus positive Wassermann and four plus positive Kahn by the Hygienic Laboratory of the State Board of Health at Columbia, S. C. This serum together with a known negative serum as control, was run through the Kahn three tube macroscopic technique, using both an antigen prepared according to the Kahn Standard formula, and the heart extracted antigen referred to above which will be described later. The result of the test was positive by both antigens in all three proportions. A microscopic examination of the precipitates formed showed slightly larger granules by the Kahn antigen, the heart extracted antigen precipitates being of smaller granules but more thickly distributed throughout the field.

In order to further test the heart extracted antigen the same two sera were run through with it by Kahn's presumptive procedure of high sensitiveness using a Kahn Special antigen for comparison. The results were identical with those of the three tube test.

In order to test the sensitivity of the heart extracted antigen the known positive syphilitic serum was run through by Kahn's quantitative procedure, using the heart extracted antigen and the Kahn Special for comparison. The serum showed a positive titer of 20 reacting units, being positive in the 1/5 dilution by both antigens, thus proving an equal sensitivity for the heart extracted antigen with the Kahn antigen. A microscopic examination of the contents of the tubes containing the next higher dilution of the blood serum showed no evidence of any precipitate by either antigen.

It would, therefore, seem that Mr. Leiboff's statement is in error, and that the heating of the beef heart tissue or of the alcoholic extracts used as antigens does not permanently destroy their precipitate forming property. Mr. Leiboff's conclusions were based on experiments with the Sachs and Georgi reaction, and my findings may therefore be said to be only further evidence of the fact which I believe is now generally admitted, that Doctor Kahn has come closer to achieving a rational precipitation technique than any of his predecessors.

The heart extracted antigen referred to, which I have called the Penn antigen after its originator, Mr. R. H. Penn of the Laboratory Staff of the South Baltimore General Hospital, Baltimore, Md., is prepared as follows: 50 grams of dried, ground and pulverized fresh beef heart is extracted for three days in the refrigerator by ether, in a 500 cc. Erlenmeyer flask, the ether being added to cover the heart tissue to a depth of one inch, and discarded at the end of each day and replaced by a fresh portion of ether. The heart

tissue is then again dried in air until all trace of ether is removed and extracted with ethyl alcohol, 5 cc per gram of beef heart by boiling gently for one hour in a flask over a Bunsen flame, with an inverted funnel in the mouth of the flask to serve as a condenser the loss of alcohol by evaporation being replaced, and further extracted by heating for one hour in a flask immersed in a bath of boiling water. The alcoholic extract thus obtained is cholesterolized to the extent of 4 mg of cholesterol per cc of extract. The antigen thus prepared is one showing usually a very high titer for the Wassermann test, in which it has been used satisfactorily now for some time at the South Baltimore General Hospital. As titrated by the writer for the routine 3 tube Kahn test it showed best results in proportions of 1 plus 25 with physiologic salt solution. As used for the Kahn quantitative procedure experiment referred to above an arbitrarily chosen titer of 1 plus 15 was employed with apparently satisfactory results.

(Signed) ARTHUR T BRICE JR BA

Florence S C, August 9 1921

Sir—It appears to me that the different results obtained by A T Brice, Jr and myself are due to two factors one is the difference in the technique of the preparation of the antigen, and the other is as Mr Brice suggests, the use of a different method in performing the tests.

Since no statement to the contrary is made, I presume that in the 'Penn' antigen the heart muscle is dried at room temperature. In the antigen which I used the tissue was treated at high temperature (JOUR LAB AND CLIN MED 1925 vi, 127) the tissue was mixed with water and autoclaved at 15 pounds pressure for ten minutes, it was then strained through cloth, washed with hot water and dried in an electric oven at 100 C until perfectly dry. The dry powder was ground completely extracted with ether and finally with boiling alcohol. Since I have not tried the Kahn test with this antigen, I am in no position to say how it would behave in that test.

The variations in the technique in the performance of the Sachs Georgi and the Kahn tests as to the method of preparing the antigen, dilution and incubation are factors which would greatly modify the results particularly since we are dealing with colloidal substances.

That heat produces a deleterious effect upon the antigen used in the Sachs Georgi test is indicated by the following statement of Sachs (Arch f Dermat u Syph, 1921 xxxii, 18) "mit den Vorwärmen bei höheren Temperaturen unspezifische Reaktionen, die nach zweistündigen Aufenthalt in Brutschrank und nachfolgender Zimmertemperatur auftreten, sukzessive schwinden. Jedoch sind sie erst nach zweistündigem Vorwärmen bei 60 vollständig eliminiert, wobei aber die Reaktionsstärke schon immerhin deutlich abgenommen hat."

The loss of precipitate forming property by incubation at 50 C cannot be ascribed to an alteration of the serum for all sera are inactivated at 56 C and it was further brought out by Kahn, Johnson and Boyd (Jour Infect Dis 1921 xxix 639) and by Kahn and Johnson (Ibid, 1922, xxxi 438) that heating at 56 C for three hours had very little effect on the reactive substances in syphilitic serum. The indication is that Sachs' results were due to some alteration of the antigen by the heat.

My interest in the Sachs Georgi test was not per se but in the question of whether complement fixation in syphilis did or did not depend upon the formation of a precipitate. I did not make use of the more delicate and more specific test of Kahn for the only reason that this work was started in 1921 before the Kahn test had gained its present popularity.

In the article referred to by Brice, on page 862, I made the following statement: "Failure to notice a precipitate macroscopically does not prove the absence of an invisible precipitate. We know that albumin particles may aggregate into larger particles without causing precipitation, provided the excess of one of the precipitate forming colloids acts as a protective colloid. Thus, further evidence is necessary to settle this question."

(Signed) S L LEIBOFF

New York, Oct 12, 1921

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

BLOOD, STAINING OF *Technic for Staining of Blood Preparations by Giemsa's Method, Van Walsem, G. C. Nederl. Tijdschr. v. Geneesk., January 23, 1926, 1, 351*

From a study of factors concerned with the production of inconstant results obtained with Giemsa's blood stain van Walsem makes the following recommendations for its use

- 1 The stain is best prepared in small quantities so as to be relatively fresh when used
- 2 To prevent agglutination and facilitate staining the blood should be collected as described below

A "separating fluid" of the following formula is prepared

Chloral hydrate 4 per cent—8 parts
Cocaine hydrochloride 6 per cent—3 parts
Morphine hydrochloride $1\frac{1}{2}$ per cent—2 parts
Glucose 2 per cent—13 parts

This solution is clear and stable. To seven parts add two parts of 0.5 per cent sodium carbonate solution. The resulting mixture, which is the "separating fluid," is turbid. One part of this solution is mixed with two parts of blood and smears made from the sediment after centrifugation.

3 After standing eight hours the slides are fixed for fifteen minutes in methyl alcohol, dried by centrifugation, immersed in 1 per cent chloroform for one minute, in methyl alcohol, and again dried.

4 Stain two hours

5 Change to dilute stain (1 drop to 1 cc) for one hour

6 Rinse, dry and differentiate two minutes in equal parts of cedar oil and oil of cloves

7 Blot dry with filter paper and examine

LIVER FUNCTION *Rose Bengal in Functional Examination of the Liver, Fliessinger, N., and Walter, H. Bull. et mem. Soc. Med. d. Hop. de Paris, April 1, 1926, 441, 525*

After an examination of more than fifty cases, the authors arrived at the following conclusions:

The rose bengal test of liver function is a simple and easy method and is harmless.

It is a test which, while it does not permit the determination of the amount of insufficiency, is always very positive in marked affections of the liver and is negative in individuals with a normal liver. For this reason it is often a valuable aid in diagnosis. A finding of more than 3 mg. per liter of blood indicates the necessity of searching for an affection of the liver which frequently is latent.

From a point of view of prognosis, it is not of immediate value except as a simple function test when used along with tests of other functions.

When repeatedly made on one patient during the course of his disease it permits the exact determination of an affection of the liver parenchyma.

DIPHTHERIA Pergola's Nutrient Substratum for Bacilli of Diphtheria Nyfeldt, A. Ugeskr f Laeger, February 18, 1926, lxxxviii, 165

Nyfeldt conducted an extensive investigation as a result of which the suitability of Pergola's medium for *B. diphtheria* is confirmed

The formula for Pergola's medium is

Serum (ox, horse, goat)-----	50 cc
Sodium chloride solution 0.8 per cent---	50 cc
Potassium tellurite solution 2 per cent----	1 cc
Yolk of egg-----	1

This mixture is emulsified in a sterile flask and from 5 to 7 cc are placed in test tubes and coagulated as a slant for one hour at 90° C and subsequently sterilized in the autoclave

WASSERMANN The Reactivation of the Wassermann Reaction and Infections Milian, G Bull Soc Med Hop Paris May 27, 1926 xlii 795

A short time ago Dufour reported examples of reactivation of the Wassermann reaction following injection of typhoid vaccine. The author was much interested in the communication and believed that a history of this subject would be appropriate.

In 1910, the author designated under the term reactivation a phenomenon consisting in the appearance of the Bordet Wassermann reaction after antisyphilitic treatment, especially 914, when this reaction appeared to be absent previously. He applied this test to the study of syphilis in general and particularly to the determination of cure from syphilis. It cannot be denied that in a patient who has had no treatment and whose reaction is negative, if the reaction becomes positive after a short treatment it is a proof that the patient was not cured, while a persistent negative reaction is an argument in favor of cure.

Since the discovery of this reactivation by 914 endeavors have been made to find better methods by which positives could more frequently be obtained. It was found that this could be done best by an incomplete treatment consisting of one or two injections. Other antisyphilitic remedies, such as mercury, iodine and bismuth appeared to be capable of giving the same results.

But later it was found that nonspecific substances had the same power such as collargol, radium bromide and milk. Some, but not all, infectious diseases are capable of producing a reactivation. This phenomenon has been observed after herpes and scarlet fever.

Neuda has shown the influence of influenza on the Wassermann reaction. Marini has shown that typhoid fever has the same power. Other authors have found the same reactivating effect in typhoid or cholera vaccine and also in sterilized milk.

The author believes that these various agents do not cause a simple reactivation of the Wassermann reaction but that they really produce a recrudescence of the syphilis itself due to a biotrophic action of the chemical substance of the vaccine or of the bacteria on the parasite of syphilis.

WASSERMANN On the Reactivation of the Wassermann Reaction in Experimental Infections Dufour H. Oasteran A. and Eldiez B. Bull Soc Hop May 20, 1926 xlii 778

For a long time the authors noticed that the Bordet Wassermann reaction became positive in a certain number of patients who had a more or less general infectious disease. Thus many cases of hereditary syphilis show a positive reaction after a tuberculous pleurisy or an epidemic encephalitis.

Cases of cerebral tumors have been reported in which the Bordet Wassermann reaction in the cerebrospinal fluid was positive. Such facts have led investigators to throw doubt on the Bordet Wassermann reaction or to consider its presence as a proof of reactivation in latent syphilis.

Milian has shown that scarlet fever, herpes, rays, etc., reactivate the Bordet Wassermann reaction in syphilitics or hereditary syphilitics.

The authors have endeavored by means of a local inflammation to reactivate the reaction in known syphilitics who had been negative. They used fixation abscesses but were not successful in producing a reactivation.

They then determined to try an experimental general infection and for this purpose used antityphoid vaccination. Two injections of this vaccine were given at eight day intervals to a man sixty years old who had been syphilitic since the age of twenty. The Bordet Wassermann reaction was negative before the injection. When his temperature was 38°C (100.4°F) he gave a clearly positive reaction. Three weeks later the reaction again became negative. This is an experimental demonstration that an intercurrent general disease can reactivate the Bordet Wassermann reaction in a syphilitic. It should be understood that all patients do not react in this manner. For example, a syphilitic with a negative reaction retained this negative reaction in spite of an attack of tuberculous peritonitis with considerable fever.

JAUNDICE Excretion of Phenolsulphonephthalein in Obstructive Jaundice, Abramson, H. A. Arch Int Med, February, 1926, *xxvi*, 291

The phenolsulphonephthalein concentration was readily determined in the presence of the bile pigments by precipitating the extraneous pigment with an excess of saturated barium hydroxide. In urine containing bile pigments the dye is partially adsorbed during precipitation, the degree of adsorption being dependent on the concentration of pigments and total urine volume. The correction for adsorption is easily determined because the quantity of adsorbed phenolsulphonephthalein in a given specimen of urine whose pigments have been precipitated by barium hydroxide, as far as the accuracy of the test requires, is a constant within the limits of from 0 to 6 mg (0 to 100 per cent) of the dye.

When there is no correction for adsorption

1 It is preferable to give the dye intravenously but intramuscular injection is almost as suitable

2 The two hour specimen should be divided into two equal parts

3 To one part an excess (urine volume plus 50 cc) of saturated barium hydroxide should be added, and this should be diluted to 500 or 1000 cc and then filtered. A portion of the filtrate should be compared with standards. If the 500 cc dilution is used, the reading is direct. If 1000 cc is the dilution, the two hour excretion is the reading times two. It is, of course, only necessary to catch a few cc of the filtrate. The dilution to 2 liters aids in colorimetric comparison.

4 If the total two hour percentage of excretion of dye is normal or only 5 or 10 per cent below the lower limits of normal, the reading stands as the excretion of the dye and the excretion may be considered normal.

When correction for adsorption must be determined

5 If the reading obtained is below normal, it must be determined whether the diminished excretion of the dye is actual or is due to adsorption. To the remaining half of the urine, of which the dye content has been determined, 0.25 cc (500 cc dilution) or 0.5 cc (for 1000 cc dilution) of phenolsulphonephthalein should be added. With intense jaundice and large urine volume the latter is preferable. The percentage of dye in this control should be redetermined.

6 The second reading in Paragraph 5 minus the first reading in Paragraph 4 gives the quantity of dye not adsorbed. For example, if the first reading (apparent two hour excretion) had been 20 per cent and if to the remaining portions 0.25 cc of the dye had been added, the second reading, granting that none had been lost by adsorption, would be 20 per cent plus 50 per cent (0.25 cc of dye to 500 cc) or 70 per cent. If the reading were 50 per cent instead of 70 per cent, it would be evident that the added dye gave only an additional 50 per cent minus 20 per cent, or 30 per cent of color. Hence, three fifths of the dye added was determined and two fifths lost by adsorption.

7 The final corrected reading of excreted dye is equal to the first reading divided by the fraction not adsorbed, or

$$\frac{20 \text{ per cent}}{\frac{3}{5}} = 20 \text{ per cent} \times \frac{5}{3} = 33 \text{ per cent}$$

In the event that the colorimetric comparison is not good because the pigment has not all been removed, practically the lost traces may be precipitated by making a second dilution using barium hydroxide instead of water as the diluent. The second dilution does not cause much adsorption of dye because the mass of precipitate is usually small. Furthermore the colorimetric comparison with standards above 30 per cent has been difficult, and the most accurate readings are obtained by comparison with lower values and by then multiplying by the necessary correction factor.

Summary of Results and Comment—The phenolsulphonephthalein excretion was found to be normal in fifteen cases of obstructive jaundice with a varied etiology. In all of these cases there was no evidence of renal insufficiency. The duration of jaundice was up to six months. Seven cases had periods of intense icterus lasting more than one month. Three of these lasted more than two months. The ages ranged from twelve to seventy-two years.

It would seem from the morphologic changes in the kidney in jaundice that the degenerative processes are to be found chiefly in the convoluted tubules. In uncomplicated obstructive jaundice, the excretion of phenolsulphonephthalein is normal. This normal excretion of the dye would fit in with the hypothesis that the excretion takes place through the glomeruli of the kidney, since there is apparently no evidence that the severe renal poisons that are present in jaundice influence the structure of the glomeruli.

These prolonged cases of jaundice demonstrate that in uncomplicated obstructive jaundice kidney function as estimated by the foregoing methods is probably unimpaired. What is the influence, then, of obstructive jaundice on diseased kidneys that are functioning normally but whose reserve is near the point at which additional injury may produce diminished function? Are the cases of obstructive jaundice which develop uræmia those in which a further slight kidney injury leads to renal dysfunction? And finally the question arises, Is this injury simply due directly to the action of bile pigments and salts on the kidney, or is it due to a disturbance of the total metabolism?

Conclusions—1 The excretion of phenolsulphonephthalein by the kidneys in obstructive jaundice has clinical and physiologic significance.

2 In fifteen cases of uncomplicated obstructive jaundice the excretion of phenolsulphonephthalein was found to be normal.

SMALLPOX The Blood in Purpuric Smallpox Ikeda K. Jour Am Med Assn June 13, 1926, lxxxiv, 1807

A clinical review of forty-eight cases. The principal changes were found in the cellular elements of the blood, involving the numerical values of the platelets and leucocytes and the morphologic structure of the leucocytes and erythrocytes.

A marked and progressive thrombopenia until its termination characterizes the purpuric smallpox in contradistinction to a rapid and steady rise in platelets after the vesicular stage in all other forms.

Another significant finding is a rapid decrease in the polymorphonuclear neutrophiles. All observers agree as to the presence of a lymphocytosis in smallpox.

Along with the appearance, in varying numbers, of myelocytes, young leucocytes and atypical and pathologic lymphocytes an absolute lymphocytosis of moderate degree was found in all forms of pustular smallpox.

In the purpuric type, however, a disappearance of the polymorphonuclear elements was effected so suddenly and so nearly completely that, with a very high leucocyte count, a leucemic state or acute benign lymphadenosis might be diagnosed from the blood alone.

Another striking condition was the appearance of pathologic forms of normoblasts, basophilic stippling, and polychromatophilia without clinical or laboratory evidence of severe anemia or chronic sepsis. These findings may be taken as indicative of intense bone marrow stimulation.

A most striking finding in the stained smear was the presence of fragments of condensed nuclear bodies, usually surrounded by a small portion of neutrophilic cytoplasm. These were derived from polymorphonuclear leucocytes.

These changes are held to be due to the direct effect of a toxin virus on the cellular structure of the mature leucocytes in the circulating blood and Ikeda believes them reasonably specific and occurring sufficiently early to make them of positive value in early diagnosis.

In the terminal stages an intense bacteriemia—frequently of hemolytic streptococci—was frequently encountered.

The hemoglobin and red cell count were practically normal.

Lymphocytes showed the same picture as seen in pustular smallpox, the presence of pathologic forms such as atypical lymphocytes, plasma cells, Turck's "irritation forms," and occasional immature forms. Monocytes showed no characteristic changes.

Myelocytes and metamyelocytes were frequently found. Eosinophiles and basophiles showed no changes. Erythrocyte fragility remained normal. Bleeding time seemed prolonged and coagulation tests were inconstant, though these observations were not recorded in a sufficient number of cases to permit conclusive impressions.

MEDIA A Synthetic Food Medium for the Cultivation of *Drosophila*, Pearl, R. Jour Gen Physiol, March 20, 1926, 11, No. 4, p. 513

The following medium has been found to be well adapted to the cultivation of the fruit fly.

Solution A.	Cane sugar	500	gm
	$\text{KN}_2\text{C}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$	50	gm
	$(\text{NH}_4)_2\text{SO}_4$	12	gm
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3	gm
	CaCl_2	15	gm
	H_2O to make 3000 cc of solution		

Solution B	Agar agar	135	gm
	Tartaric acid ($\text{C}_4\text{H}_4\text{O}_6$)	30	gm
	KH_2PO_4	6	gm
	H_2O to make 3000 cc of solution		

Melt the agar thoroughly in the water with heat, add the salts, and for the medium to be used in the fly bottles, mix equal parts of Solutions A and B. For some kinds of work it has proved desirable to have the final food a little less stiff, in which case a smaller amount of agar is used, without changing the composition otherwise.

This medium has a pH when freshly made, cooled, and the agar set, of approximately 3.7. As the flies live upon it the pH falls to a value of 3.0, or in some cases even lower.

STAINS Chemical Studies on Polychrome Methylene Blue, MacNeal, W. J., and Killian, J. A. Jour Am Chem Soc, 1926, 48, 710

A description of methods for the manufacture of dimethylene blue chromate, methylene Azure B, methylene Azure A, and methylene violet, for the details of which the original paper should be consulted.

TUBERCULOSIS Blood Cell Volume Index in Pulmonary Tuberculosis, Newcomb, P. B. Southwestern Med, March, 1926

Using the Van Allen hematocrit, Newcomb reports a study of the blood cell volume in 108 cases of pulmonary tuberculosis as a result of which it is concluded that the blood cell volume index is advocated as an addition to the routine blood examination in pulmonary tuberculosis. It appears to be less liable to human error than the hemoglobin percentage and color index in that its computation is mathematically and mechanically controlled.

In a brief experience the blood cell volume index has been found to bear generally a true and consistent relation to the patient's condition—normal or high readings being the rule in those who are showing progress under treatment while low values are recorded for patients who are failing physically.

BILIRUBIN Comparison of the Sensibility of the Reactions of Gmelin and Grimbart for the Presence of Bilirubin in the Urine Garnier M and Giroire H Paris, Med, May 13, 1926 Vol 48

Gmelin's reaction is much less delicate than the precipitation methods, especially that of Grimbart the technique of which is as follows: 5 cc of a 10 per cent solution of barium chloride is added to 10 cc of urine the mixture is shaken violently and is then centrifuged. The precipitate consisting of sulphate phosphate and bilirubinate of barium is first washed by centrifuging in 10 cc of water then after decantation it is taken up in 4 cc of 90 per cent alcohol containing 1 per cent of its volume of pure hydrochloric acid. This mixture is placed on a boiling water bath for about one minute. If the liquid is colorless there is no biliary pigment in the urine but if colored green or bluish green the urine contains bilirubin, if it is colored blue violet or carmine red—the urine contains biliary pigments in the process of oxidation. Finally if the liquid presents a brownish tint two drops of hydrogen peroxide solution must be added and the mixture again is placed on the boiling water bath the appearance of a green color shows the presence of biliary pigment.

Grimbert's reaction is ten times as sensitive as Gmelin's reaction.

In certain urine Gmelin's reaction is obtained as the colors are masked by other colored substance in the urine but Grimbart's reaction is positive if biliary pigments are present.

SPORE STAIN A Safe Spore Stain for Glass Use May H G Stain Technology, July 1926 Vol No 3, p 105

- 1 Make film and fix by heat as usual
- 2 Cover with small amount of 5 per cent chromic acid
- 3 After thirty seconds add about twice as much concentrated ammonia as there is chromic acid on the slide. Allow to act about two minutes
- 4 Rinse with tap water
- 5 Steam with carbol fuchsin for two or three minutes
- 6 Rinse
- 7 Destain with one per cent sulphuric acid for fifteen to thirty seconds
- 8 Rinse again and flood slide with the tap water
- 9 Add to this a few drops of Loewy's methylene blue and allow to stain for ten to thirty seconds
- 10 Rinse, blot, dry and examine

The dilution of the methylene blue is necessary because the pre-treatment increases the staining properties of the cells so much that a good methylene blue overstains when used in concentrated form even if rinsed off a second or so after it is put on the slide.

The results with this method will not always be equally good as a lack of sufficient destaining may leave some vegetative cells red or an overstaining with methylene blue will make the cells opaque and even form a heavy film over some of the spores but the spores will always be stained and clearly visible. The average student has no more difficulty with this method than with the Gram stain and usually obtains more reliable results.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*Medical Laboratory Methods and Tests**

THE purpose of this book is to present, for the use of the physician, the chemical, microscopic, and other examinations which may be made in the office laboratory

Many of the procedures are, quite naturally, those in vogue in Europe, although the work of American investigators is not neglected, and the work should serve very well for the purpose for which it is intended

Forensic Medicine and Toxicology†

THE increasing importance of medical evidence and the fact that any physician may find himself called as a witness renders some knowledge of medicolegal procedure a necessity. Such a survey is presented in this ninth edition of Buchanan's work.

The book is divided into two sections, the first concerned with forensic medicine, and the second with toxicology.

While the legal procedures are those of the English courts, the book should prove useful to American readers because of its clear presentation of the subject.

A Statistical Survey of Three Thousand Autopsies‡

A REPORT of a study of the postmortem examinations performed during the years 1900-1923. None but fairly complete autopsies are included and the material surveyed is exhaustively classified and cross indexed.

The report is divided into nineteen sections, each concerned with particular conditions and is embellished with numerous tables and charts.

It illustrates anew the striking importance of congenital syphilis as a cause of infant mortality (112 of 307 cases).

The first three sections are concerned with a general survey, the remainder discussing the conditions found in seriatim.

The relative frequency with which various conditions were encountered is shown below.

*Medical Laboratory Methods and Tests. By H. French. Physician, Guy's Hospital and T. Nuttall, Assistant, Guy's Hospital. Cloth. Pp. 246. 62 illustrations and 3 colored plates. Price \$2.50. Chicago Medical Book Co.

†Forensic Medicine and Toxicology, ed. 9 (Buchanan) revised and enlarged by J. E. W. McFall. Professor in Forensic Medicine and Toxicology, University of Liverpool. Cloth. Pp. 445. 57 illustrations. Price \$5.00. William Wood and Co.

‡A Statistical Survey of Three Thousand Autopsies. By Wm. Opuls. Department of Pathology, Stanford University. Paper. Pp. 370. 16 charts. Price \$2.50. Stanford University Press.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Tuberculosis	64 %
Septic infections	42 %
Arteriosclerosis nephritis	37.5 %
Gonorrhea syphilis	31 %
Tumors	30 %
Malformations	14.5 %
Miscellaneous lesions	40 %
Calculi	11 %
Alcoholism drug addiction	9.5 %
Injuries poisonings	10.6 %
Acute infections	6.6 %
Cirrhosis naemia	10 %
Diseases of metabolism	13.8 %
Animal parasites	1.75 %

The enormous amount of material thus gathered and minutely studied should be invaluable to pathologists and those interested in disease

*Diseases of Children**

IN HIS prefatory remarks Cameron defines his handbook as one that deals briefly with the fundamental principles involved in the application of the fundamental and primary conceptions relating to pediatrics. This presentation makes no pretense of being a complete textbook but on the contrary stresses its message to medical readers in a most impressive, brief and practical manner. The book is more or less devoid of theoretical discussions laboratory procedures or microscopic and gross pathology. It presents in a practical manner the salient features of certain common disorders met with in infancy and childhood as viewed and interpreted through the eye of the pediatric clinician. The wealth of condensed and common sense observations are presented in a somewhat similar manner as found in his splendid and famous book *The Nervous Child*. Cameron deplors the lack of a more profound and intensive teaching of pediatrics in the routine medical curriculum of schools and colleges and when one takes into consideration that approximately 30 per cent of the average physician's patients are within the age limits of infancy and childhood certainly this criticism is amply justified. The subjects of the thirteen chapters were chosen to present the most important everyday problems that confront the physician in the routine course of his medical duties and it is with this view that Cameron's vision and broad knowledge are interpreted within the pages of this small textbook.

The chapters are captioned as follows:

The Study of Disease in Childhood
 Diseases of the Newly Born Congenital Traumatic and Infective
 Breast Feeding and Its Management
 Sleeplessness and Nervous Unrest in Infancy
 Catarrhal Infection.
 Vomiting in Infancy
 Diarrhea in Infancy
 Constipation
 Inherited Predisposition to Disease
 Conduct and Management and Their Effect Upon Health
 Some Surgical Operations Upon Children
 Backwardness and Convulsions in Infancy
 Diet

As a medical handbook the features and procedures that are chosen are so well and clearly presented that it is indeed worth while to absorb Cameron's broad clinical experiences in the several chapters of this booklet.

Diseases of Children By H. C. Cameron Physician in Charge Department for Diseases of Children Guy's Hospital. Cloth Pp 199 Oxford University Press 1936

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EDITORIALS

The Prophylaxis of Acute Infections

QUARANTINE as a prophylactic measure is, perhaps, one of the oldest means utilized in the prevention of disease, as it is mentioned in Biblical writings, referred to in some of the earliest known hieroglyphic records and, perhaps, under the guise of "taboo" may be recognized among the antiquities of primitive peoples

Originally applied without distinction to disease in general, the manner and duration of quarantine has undergone various modifications in modern days in accordance with the evolution of understanding of the etiologic agents and the manner of their dissemination in various diseases, and recent studies suggest some further modification of the value of the method as applied to certain specific infections, such as scarlet fever

Holst¹ has recently published a study of the relation of quarantine to the incidence of scarlet fever in Norway which, while not final in its conclusions, at least opens up an interesting vista and suggests the advisability of the collection of similar and more extensive data in other countries

In Norway scarlet fever is treated largely by isolation in the home or hospital, and a study of the incidence of the disease reveals the lowest inci

dence where living conditions and the absence of hospitals have always made the fight against the disease difficult, and the highest incidence where the facilities are the best

That this is not due merely to incompleteness of notification is shown by the fact that in Bergen representing the first situation, but where notification is as effective as in Oslo representing the second situation, the incidence is much lower than in the last named city

Statistics quoted by Holst covering 21 cities and the country district for the years 1880 to 1919 show that it is impossible to prove statistically that the disease can be eradicated by isolation, but the same statistics also show that, whereas in the early eighties scarlet fever was one of the most important causes of death, in later years it becomes relatively unimportant

What is the relation of quarantine, if any, to this fact?

According to Chapin isolation by the segregation of grave cases due to a virulent strain of the virus is the reason for the lessened mortality, but Holst, from a comparison of the statistics of Norway and Sweden, points out that the decline in the Norwegian mortality curve was most marked when hospital isolation was still very incomplete and in those parts of the country where it was most difficult to control the spread of the disease. Holst, therefore, cannot agree with Chapin's conclusion and maintains that the incidence of scarlet fever is dependent upon conditions still unknown and that it has not been perceptibly influenced by quarantine measures

He comments upon these facts

1 Practical experience shows that scarlet fever is frequently transmitted by contact

2 The not infrequent absence of any source of infection suggests the possible existence of a carrier

3 There is no definite knowledge as to the period of infectivity

All these facts in sum affect the rationale of quarantine and modify its duration experience showing that some convalescents are harmless after three weeks while others are still dangerous after six weeks

The quarantine in hospitals of grave cases with mild cases which are thus exposed to grave complications has also been objected to on this ground, and the undoubted fact that many mild cases are unrecognized until the period of desquamation has led, in Bergen since 1910 to the exclusion from school of contacts for a period of only five days—a procedure apparently without effect upon the incidence of the disease

In two Norwegian cities—Bergen and Trondhjem—scarlet fever has been treated since 1910 without isolation and without disastrous results. Houses are placarded and prompt notification is insisted upon, and while the other children are kept from school for five days they are allowed to play in the street after desquamation is completed the patient's room is cleaned but no further precautions are taken. A comparison of the cases per thousand in Bergen and in Oslo, where strict hospitalization is practiced, shows a constantly lower rate for the former city from 1900 to 1922

Holst concludes therefore that the abolishment of isolation has had no influence upon the incidence of scarlet fever, but he is uncertain as to the

effect upon the case mortality. He believes that it is essential to keep in touch with the disease because malignant or fulminating characteristics may appear at any time.

Reference has been made to the variability of the period of infectivity during convalescence from scarlet fever and the studies of Gordon³ upon the presence of hemolytic streptococci in the upper respiratory tract of scarlet fever convalescents are of distinct interest in this connection.

The apparently normal convalescent carrier is, obviously, of essential importance but, despite this fact, little is known of this type. Gordon comments upon the variability of opinion as to the period of infectivity and the great value of a method of cultural control in scarlet fever similar to that in diphtheria and reports his studies of 200 cases over a period of eight months from which he draws the following conclusions:

The severity of the disease definitely influences the time that hemolytic streptococci remain on the mucous surfaces of the upper respiratory tract, being less for mild than for moderately severe, and still less than for severe cases.

The administration of antitoxin apparently influenced the frequency of positive cultures, the incidence being 38 per cent as opposed to 64 per cent in cases not receiving serum.

Age and sex were without apparent effect while complications definitely influenced the incidence of positive cultures, serum-treated cases without complications having 32 per cent, and those with complications 51 per cent of positive cultures, and the rate for control cases being 58 per cent without and 75 per cent with complications.

Gordon believes, therefore, that complications account for chronic carriers and that a severe uncomplicated case is safer for release than a mild case with complications.

In positive cases the organisms are most commonly found in the throat only, about one-third show organisms in both throat and nose, and only rarely is only the nose positive. The highest percentage of positive cultures occurs in the age group in which complications are most frequent.

From his studies Gordon believes that consideration should be given to the practicability of a revision of the existing regulations for isolation in scarlet fever and that some variation in time be governed by cultures for scarlet fever streptococci.

As a corollary to these studies Harmon and Perkins⁴ have analyzed the data concerning the relation of latitude to the seasonal occurrence of scarlet fever, and also of diphtheria and measles in the United States and in Australia, emphasizing the time of year in which the maximum and minimum incidence of these diseases occurs, rather than the total number of cases.

Their statistics covering the years 1913 to 1924, inclusive, show that, in the United States, November, May, and January are the months of maximum, and July, September, and August, the months of minimum incidence for the diseases in question, the peak of seasonal prevalence being from one to three months earlier in the South than in the North, these tendencies being observed in years of both low and high prevalence.

While the analysis of their material suggests that latitude has an effect upon the seasonal distribution of the prevalence of these diseases, the degree to which temperature, moisture, sun's rays or other factors are concerned awaits the accumulation and analysis of further data which, it is to be hoped, may be furnished eventually through the labors of the League of Nations

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—R A K

The Doctor's Bookshelf

THE doctor, of all men, must be a lover of books, for reading, says one of the ancients, "maketh a full man"

He must, of course be a reader and a student of medical texts because medicine is a changing science always in a state of evolution, the theories of today being proved or disproved tomorrow

The tendency of his medical library naturally will be to concentrate itself around the subjects which constitute his predominant interest but there will be, if he is to be well grounded and well rounded in his profession, a judicious sprinkling also of those works which include allied subjects and those contiguous to and inseparable from his particular interest

The necessity for this is obvious for his subjects are human beings, and human beings are not hearts, lungs stomachs, and various other separate anatomic entities, but complex and intricate beings composed of innumerable structures welded into one inseparable and interlocking whole

There will be, first of all, works on the causes and mechanism of disease production, and these should show honorable scars of frequent use, for, if one were restricted to one field of study only, it would be most valuable to know the pathology, that is, the mechanism whereby functional disruption, which is disease, occurs

The care with which his patients are studied and their symptoms interpreted will be reflected in that part of the bookshelf which pertains to diagnosis and the means and methods whereby it may be achieved In that part of the library pertaining to treatment the most valuable sources of information will be found in the doctor's case records which are really, after all, the tomes in which are writ the lessons of experience

It is better to have a small library and to read it than to have a large one mainly to look at

Every man interested in his work will, sooner or later, begin the collection and filing of pertinent reprints and data The completeness with which this is done will reflect the study and survey of the current and sometimes ephemeral literature of journals The manner of its doing is a matter of personal preference but should be devised to render the data accessible

Doctors are busy men and many there are who "have not time to read" This is hardly a valid excuse. A famous Practise of Medicine is said to have been written largely in the short interval between breakfast and the morning office hours. There is always time to do what one wants to do.

It is quite difficult to read a textbook from cover to cover, and doubtful if the information absorbed is worth the labor, but many a case has a puzzling feature, a phase concerning which recollection is faulty and which may be looked up profitably, even when one is busy. And such a practise leads to much reading in the long run.

It is impossible, of course, to carry the contents of one's bookshelf in one's mind, but it is possible to know, without haphazard search, where to go for specific information and how to utilize it when it is found.

Books must be read to be useful. A medical library, unlike others, is ephemeral. The fame of the author is not always a true index to the true worth of his book but, in the main, it warrants at least an examination of the work. It is one thing to have knowledge and another to have the ability to impart it.

Monographs are sometimes of more present value than ponderous sets, sometimes, also, more likely to be read.

It is well not to buy solely because the work is new, be sure the subject is of sufficient interest to tempt you to read, and the author capable of his subject.

At least one authoritative work on the history of medicine should be included and read.

The medical library will, of course, be a part of the workshop and its armamentarium and not the least valuable.

There will be, without doubt, other books—the classics, and those concerned with particular interests and special hobbies.

The doctor's interest in and choice of literature will prove a shrewd index to his personality. An interest in literature is allied to an interest in his profession for he must be a student of human nature, and in the classics he will find it dissected and portrayed, he must appreciate good writing if he is to do any, even a case history to be truly valuable should be well written, and in his books he will find examples to admire and profit by.

The doctor must know how to express himself if he is to impart his thoughts to others by word or pen, and from his books and reading he will draw his vocabulary and learn how to marshal his words and sentences into companies and divisions to carry the trenches of opposition or ignorance by irresistible assault.

And, in the learning, when the day's work is done and the doctor sits in the quiet shadows, living, perhaps, in other days, he will find pleasure, relaxation, and contentment, and in the companionship of his bookshelf find his other self, coming forth renewed and so refreshed in body, with the facets of his mind young and ready for the new.

—R A K

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THE BLOOD PICTURE IN PURPURA*

By NATHAN ROSENTHAL M.D. NEW YORK CITY

INTRODUCTION

THE complete blood examination is of paramount importance in the diagnosis, prognosis, and treatment of all cases showing purpura either as a predominant or secondary symptom. It is indeed surprising that (except in France) little attention has been paid to the blood changes until recently. The early reports of the diminution of the blood platelets in purpura by Brohm (1881), and L. Krauss (1883) in Germany, were ignored until Glanzmann¹ (1916) revived them. Denis² (1887) and Hayem³ also reported similar observations on the blood platelets. Denis found a normal clotting time of the blood, and Hayem made the important observation of the absence of clot retraction and transudation of serum. Hayem⁴ and his pupils (Bensaude Rivet and others) deserve great credit for the preciseness of their studies of the main hematologic changes in purpura. Duke⁵ noticed the prolonged bleeding time from the lobe of the ear and put this test on a working quantitative basis. Although this type of bleeding time may be prolonged in purpura, Leschke and Wittkower⁶ point out that the operative bleeding time may not be unduly increased. Giffin and Holloway,⁷ Leschke and older observers have seen patients with purpura stand major operations. Waltner⁸ and Wittkower⁹ have reported cases of uncomplicated labor in purpuric women. In both cases the newborns were also purpuric. Exsanguinating hemorrhages have followed tonsillectomy and childbirth in two cases in our series and required numerous transfusions to keep them alive. Artificial purpura can be brought about by the application of a tourniquet to the arm

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, Washington, D. C., May 13, 14, and 16, 1928.

From the Medical and Pediatric Divisions and the Laboratories of Mt. Sinai Hospital, New York.

This was first noticed by Gioeeco¹⁰ and used later by Hess¹¹ as one of the main tests for purpura. This is called the tourniquet or capillary resistance test. In the Hess test the arm is compressed at 90 mm of mercury for three minutes. Lewis¹² recently suggested the use of the sphygmomanometer at 70 mm of mercury for three minutes, but this method shows a greater number of positive results than the ordinary rubber tourniquet which was used in the study of our cases. The greatest impetus in the study of purpura was given by Frank¹³ when he published his theory concerning the myelotoxic influence of the spleen on the bone marrow. He introduced the term essential thrombopenia (diminution of the thrombocytes or blood platelets), which describes the main hematologic feature of purpura. Kaznelson¹⁴ (1916) reported the first successful case of purpura treated by splenectomy, this was done as a result of his belief that the spleen was a destructive agent of the blood platelets producing thrombocytopenia. For that reason he used the name thrombolytic purpura.¹⁵ Since then, numerous cases of purpura have been cured by splenectomy.

CLASSIFICATION OF PURPURA

The first classifications of purpura were mainly clinical (Henoch,¹⁶ Litten¹⁷). It was thought that all purpuras were about the same, differing only in their intensity. Purpura which was limited to the skin was called purpura simplex and where mucous membranes were also involved, it was called purpura hemorrhagica, purpura with additional joint involvements was called Schoenlein's disease, purpura associated with intestinal hemorrhages was called Henoch's purpura. Havem,⁴ Lenoble,¹⁸ and Hutinel¹⁹ have made classifications of the purpuras, according to the hematologic changes, which are more helpful. The first modern classification was made by Glanzmann,¹ who divided the purpuras into two groups (1) anaphylactoid and (2) morbus maculosus Werlhofii. Recent classifications are those of Frank²⁰ and of Guglielmo²¹, the latter classification is as follows:

Guglielmo's Classification

- I Morbus Maculosus Werlhofii
 - (b) Acquired Form, Acute or Chronic
 - (c) Constitutional Form, Chronic
- II Schoenlein-Henoch's Purpura or Pechosis Rheumatica
- III Symptomatic Purpura Hemorrhagica
 - (a) Diseases of the Blood (Myelomas, Aplastic, Pseudoplastic, Hypoplastic, Hyperplastic, Aleukia, Hypoleukia, Splenic, etc.)
 - (b) Endotheliosis
 - (c) Infectious Diseases, Avitaminosis, etc.
- IV Hemophili
- V Pseudo-hemophili (Fibrinopenia)

The following classification is based on a clinical and hematologic study of 172 cases:

A. Purpura as a Result of the Diminution of the Blood Platelets or Thrombocytopenic Purpura

- 1 (a) Acute Thrombocytopenic Purpura (death 6) (Table I)
- (b) Acute Thrombocytopenic Purpura (well 3) (Table I)

- 2 Chronic Thrombocytopenic Purpura 47 cases
 - (a) Normal clot retraction, 4 cases (Table II)
 - (b) Slight clot retraction 11 cases (Table III)
 - (c) No clot retraction 32 cases (Tables IV, V and VI)
 - 3 Chronic Aplastic Anemia 5 cases (Table VII)
 - 4 Acute Aplastic Anemia 1 case (Table VII)
- B Symptomatic Thrombocytopenic Purpura**
- 1 Leucemia
 - (a) Leucopenic Myeloid, 18 cases (Table VIII)
 - (b) Leucocytic Myeloid 13 cases (Table IX)
 - (c) Leucopenic Lymphatic 6 cases (Table X)
 - (d) Leucocytic Lymphatic 4 cases (Table X)
 - 2 Subacute Bacterial Endocarditis, 11 cases (Table XI)
 - 3 Splenomegaly
 - (a) Resembling Banti's Disease, 8 cases (Table XII)
 - (b) Gaucher's Disease 7 cases (Table XIII)
 - (c) Acquired Hemolytic Icterus 1 case
 - 4 Pernicious Anemia 2 cases (Table XIV)
 - 5 Tuberculosis 4 cases (Table XV)
 - 6 Drugs
 - (a) Quinine, 1 case
 - (b) Salvarsan 1 case
 - 7 (a) Carcinoma 2 cases
(b) Dermoid Cyst of Ovary 1 case
 - 8 Typhoid Fever 1 case
- C Chronic Thrombasthenic Purpura, 2 cases**
- D Hypertension and Nitrogen Retention, 6 cases (Table XVI)**
- E Jaundice 4 cases (Table XVII)**
- F Arteriosclerosis (decurv) 3 cases (Table XVIII)**
- G Schoenlein Henoch's Purpura, 9 cases (Table XIX)**
- H Purpura Fulminans 1 case**

THE BLOOD PICTURE

The following blood examinations are the most important for the differentiation of the various types of purpura which we have studied

1 Hemoglobin This may be determined in percentage by the use of the new nonfade hemoglobinometer

2 Red blood cells and white blood cells Standardized pipettes and counting chamber should be used for the enumeration of the red and white cells

3 Blood platelets There are two chief methods of determining the number of blood platelets First, 3 per cent sodium citrate freshly prepared is used as in performing the red cell count The blood platelets are counted after they have settled in the counting chamber (Ottenberg and Rosenthal) Second, by the use of citrated plasma, 0.03 c.c. of 30 per cent sodium citrate to each cubic centimeter of blood The blood platelets are counted from the plasma after the red cells settle (Thomsen,³ Grim⁴) The plasma is then diluted (1:20) with 3 per cent sodium citrate, and the platelets are enumerated in the ordinary counting chamber after sedimentation (fifteen minutes at least)

The morphology of the blood platelets should also be studied in smears or in citiated plasma. The blood platelets are usually very large in cases of thrombocytopenic purpura, pernicious anemia, and leucemia.

4 Differential count. This must be carefully done on preparations stained with the Jenner-Giemsa method (Rosenthal²⁵). This method stains the abnormal cells extremely well for their proper identification (Rosenthal²⁶).

5 Coagulation time of the blood. The Lee and White²⁷ method is the simplest and best. The blood is withdrawn from a vein for the coagulation time and for the platelet count at the same time.

6 Bleeding time. This must be done from the ear lobe.

7 Capillary resistance test. This can be done either with an ordinary rubber tourniquet or with the cuff of a sphygmomanometer, compressing the arm at 70 mm. of mercury, or at a pressure midway between systolic and diastolic, for three minutes. A positive test is shown by the appearance of petechiae after constriction.

8 Clot retraction. The blood used for the coagulation time is observed at half-hour or greater intervals. Normally, retraction begins within thirty minutes and is complete within four hours (Palmer²⁸).

Blood changes were found in a great number of purpuric cases and were often diagnostic. Clinical symptoms alone are not sufficient for the determination of the exact nature of the underlying condition unless the case has already been diagnosed, such as typhoid fever or jaundice, and the purpura appears later as a complication. One of our patients with chronic thrombocytopenic purpura which later was cured by splenectomy, first entered the hospital with frank symptoms of Schoenlein-Henoch's disease (joint symptoms and hematuria). On account of the insufficient hematologic studies, the patient was discharged as such. During a later admission to the hospital, a complete blood study showed a low platelet count, prolonged bleeding time, positive tourniquet test, and absence of clot retraction, and the proper diagnosis was then made. It was not unusual for cases of purpura hemorrhagica in male children to be mistaken for hemophilia (pseudohemophilia). We have not seen true purpuric manifestations in our cases of hemophilia.

A THE BLOOD CHANGES IN CASES OF PURPURA

1 *Acute Thrombocytopenic Purpura* (Table I).—The acute cases of purpura differ from cases of chronic purpura only in their course. There are three varieties when first seen. (1) they may run a fatal course, as in six cases reported, (2) they may recover spontaneously (apparently the first case of purpura reported by Werlhof belonged to this group), and (3) the case may be the onset of a chronic type. The blood pictures of all the acute cases are characterized by a marked diminution of the blood platelets. The diminution of the hemoglobin and red blood cells depends upon the amount of hemorrhage which has taken place. When first seen, the hemoglobin and red blood cells may be normal, but after excessive blood loss, one finds a proportionate fall in the hemoglobin and red blood cells. Case R F, Table I, had rather high hemoglobin and red blood cells on admission to the hospital, but within two weeks, the hemoglobin and red blood cells fell to very low figures. A citrate

TABLE I
ACUTE THROMBOCYTOPENIC PURPURA

NO	NAMF	HGB	RBC.	WBC	PL.	PN	L.	M	CT	BT	TT	CR
1	R F	90	4.8	8	1	54	38	5	8	25	+	None
2	C.M	88	4.1	12	1	73	21	5	19	19	+	None
3	H E	22	1.1	7	2	62	23	11	14	120	+	None
4	S W	48	3.6	12	5	80	18	2	6	80	+	None
5	A T	85	5.0	6	10	70	25	3	12	6	+	Slight
6	I G	24	1.6	8	17	79	16	5	8	190	+	Slight
7	M M	42	2.3	16	20	73	20	5	11	8	+	None
8	R G	64	3.5	12	20	59	37	4	6	60	+	None
9	P D	84	4.4	7	20	60	32	8	9	10	+	None

The following abbreviations are used for the tables

HGB—Hemoglobin RBC—Red Blood Cells (in millions) WBC—White Blood Cells (in thousands) PL—Platelets (in thousands) PN—Polynuclear Neutrophils L—Lymphocytes M—Monocytes CT—Coagulation Time BT—Bleeding Time TT—Tourniquet Test. CR—Clot Retraction

transfusion given to this patient was followed by an increased amount of bleeding. This was attributed to the citrate but the same excessive bleeding occurred after a direct transfusion.

The white blood cells are either normal in number or somewhat increased. The differential blood picture is about normal, except in two cases where the monocytes were increased to 8 and 11 per cent. The coagulation time is normal or somewhat prolonged in all cases. The special tests show the characteristic changes as a result of the diminution of the blood platelets, the normal or increased coagulation time, greatly increased bleeding time, positive tourniquet test and failure of the proper retraction of the blood clot in all cases. The blood picture in the acute cases is similar to that of the chronic cases.

2 Chronic Thrombocytopenic Purpura (Tables II, III and IV)—The cases of chronic thrombocytopenic purpura are the most numerous. The outstanding symptoms of all the cases are the hemorrhages into the skin and from the mucous membranes. Some of the cases reported here have been followed for years. The blood platelets returned to normal, and the purpura and hemorrhagic tendency disappeared in a few cases at times (intermittent purpura). As a rule the diminution of the platelets is always present. The purpuric condition in some cases is very latent and runs a mild course.

All the manifestations of this disease can be said to be due to the diminution of the blood platelets in the circulating blood. This diminution in the blood platelets is believed to be due either to a disease of the bone marrow, most likely of the megakaryocytes (the mother cells of the blood platelets), or to an increased destruction in the spleen. Frank believes in a myelotoxic influence of the spleen affecting the formation of the platelets. Jeddika and Altschuller⁹ find distinct morphologic changes in the megakaryocytes of the bone marrow stained with Giemsa. On the other hand, Kaznelson¹⁵ believes that there is an increased destruction of blood platelets in the spleen to account for the thrombocytopenia. This has been the *raison d'être* for the cure or treatment of this disease by splenectomy. In the study of 22 postoperative cases of this type of purpura treated by splenectomy, I³⁰ have come to the conclusion that both ideas are correct. In some cases the thrombocytopenia is due to a diminished formation of blood platelets in the

bone marrow, and in other cases, there is an increased destruction. In the first group, the blood platelets, after preliminary increase, return to their former low level, and in the second group, the blood platelets, after a much greater preliminary increase, return to normal figures. We may consider the splenic factor as an aggravating one in the first condition, and as a primary disease of the spleen in the second group.

The blood platelets not only vary in number in this disease but also in their quality. The most frequent blood picture encountered in this group is a normal hemoglobin and red blood cell count (or diminution comparable to the amount of hemorrhage), a normal white cell count, and a normal differential count. Leucocytosis (polynucleosis and monocytosis) may occur after hemorrhage. The blood platelets vary from 2,000 to 100,000, the average

TABLE II

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING PRESENCE OF CLOT RETRACTION

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	SG	62	3.4	7	5	66	29	5	6	12	-	Present
2	EB	69	4.7	6	48	62	27	7	8	3	+	Present
3	LM	76	5.3	10	50	70	28	2	7	3	+	Present
4	EP	98	4.8	13	80	57	40	3	12	2	+	Present

TABLE III

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING SLIGHT CLOT RETRACTION

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	ML	84	5.1	17	5	58	35	6	6	17	+	Slight
2	EH	77	3.8	11	10	59	38	3	10	8	+	Slight
3	MB	85	4.8	11	10	68	26	5	7	13	+	Slight
4	MA	67	3.4	10	20	64	31	3	5	10	+	Slight
5	LC	80	5.3	10	20	54	40	4	6	6	+	Slight
6	FD	70	5.1	9	20	68	17	14	11	30	+	Slight
7	JG	91	5.3	7	30	67	24	7	10	9	+	Slight
8	MM	114	7.5	11	30	65	22	7	5	9	+	Slight
9	AB	36	1.8	4	40	66	25	8	9	8	+	Slight
10	RA	60	3.3	5	50	68	27	4	16	5	-	Slight
11	MH	30	1.9	5	100	37	60	2	6	5	+	Slight

TABLE IV

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING ABSENCE OF CLOT RETRACTION AND PLATELETS BELOW 10,000

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	FF	66	5.4	8	2	60	30	8	8	26	+	None
2	BS	70	3.5	11	2	59	34	7	6	6	+	None
3	EG	50	2.8	6	4	84	13	3	11	10	+	None
4	MN	58	3.5	8	4	76	22		13	6	+	None
5	AG	98	5.6	6	5	80	12	8	12	16	+	None
6	MH	40	2	8	5	63	32	4	12	15	+	None
7	MS	70	3.8	20	5	71	13	8	7	10	+	None
8	SH	92	4.8	11	6	64	20	3	6	6	+	None
9	RG	38	2.2	6	6	54	26	13	10	10	+	None
10	LC	74	3.9	8	8	67	27	4	9	25	+	None
11	JW	91	4.8	8	8	71	21	6	13	21	+	None
12	EG	94	5.1	12	10	65	31	5	10	42	+	None
13	YK	86	4.2	10	10	72	20	6	4	16	+	None
14	BL	79	4.8	12	10	72	19	6	6	10	+	None
15	JL	80	5.7	10	10	45	53	2	6	13	+	None
16	SR	96	4.8	13	10	76	15	9	8	9	+	None

TABLE V

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING ABSENCE OF CLOT RETRACTION AND PLATELETS ABOVE 10,000

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	DR	64	3.1	8	20	64	37	8	8	8	+	None
2	RL	80	4.2	13	34	57	41	2	8	4	+	None
3	RB	75	4.5	11	25	72	21	3	10	10	+	None
4	MA	98	5.1	12	30	73	17	7	6	17	+	None
5	GL	98	4.6	10	30	52	42	4	8	10	+	None
6	JY	30	1.8	18	30	78	18	3	7	16	+	None
7	MO	40	2.3	15	40	91	4	5	20	8	+	None
8	CS	37	3.2	4	42	48	33	11	13	20	+	None
9	RJ	39	1.9	8	45	77	10	3	9	6	+	None
10	JC	83	5.0	14	50	68	27	3	6	6	-	None
11	HS	84	4.3	14	50	70	15	6	10	16	+	None
12	SR	70	3.0	23	80	83	13	4	5	7	+	None
13	CG	63	3.2	21	90	71	24	3	8	20	+	None

TABLE VI

CHRONIC THROMBOCYTOPENIC PURPURA WITH NORMAL BLEEDING TIME

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	CT	125	5.5	12	5	71	3	6	11	1	+	None
2	BT	75	4.1	5	5	45	2	2	12	2	+	None
3	HH	84	4.3	9	6	76	18	3	7	2	+	None

platelet count being 25,000. The bleeding time does not seem to be associated with the number of platelets. In three cases the bleeding time was normal (Table VI), although the blood platelet average was 5,500. The coagulation time is normal or slightly prolonged. The tourniquet test was present in all cases with the exception of two. The clot retraction was absent or slight in all cases except in four, in which the clot retracted normally (Table II). In one of these cases, a splenectomy was performed with excellent results, the patient later had a subtotal gastrectomy done without any hemorrhage.

We can deduce from the study of all these cases that the cause of the hemorrhagic phenomena is due not only to the diminution in the blood platelets, but also to some change in their quality. Then altered function can be determined by the special tests outlined: mainly the coagulation time, bleeding time, tourniquet test and clot retraction which correspond to the following functions of the blood platelets:

1 Coagulative function. It is beyond the scope of this paper to take up the various theories concerning the coagulation of the blood. The important relation of the blood platelets in instituting the coagulation of the blood has long been pointed out by Hayem,³¹ Salvioni,³ Bordet and Delange³³ and also recently by Tait.³⁴ This factor is apparently not involved in cases of purpura. It has been pointed out, however, that serum obtained from a blood clot poor in platelets contains less thrombin as measured by its action on fibrinogen or hydrocele fluid.

2 Hemostatic function. Hayem³¹ has pointed out the vulnerability, the viscosness and the agglutination of platelets to foreign bodies. Wright and Minot³⁵ have observed and produced the viscous metamorphosis of the platelets. Hayem had also noticed the agglutinative power of blood platelets.

and then adhesions to wounds which he terms the "clou hemostatique," the method by which hemorrhage is prevented from the capillaries. In purpura hemorrhagica this property is greatly interfered with on account of the blood platelet diminution and some change in their quality. In other diseases, such as pernicious anemia and leucemia, there is also a diminution of the blood platelets without change of quality, as a rule, so that the bleeding time may be normal, and purpuric manifestations may not occur. In purpura, however, an increase in blood platelets, even up to 100,000 and in a few cases higher still, may be accompanied by a prolonged bleeding time. The bleeding time was normal in only three cases (Table VI).

3 Tourniquet Test The tourniquet test indicates the condition of the capillaries. The appearance of petechiae at the elbow after the application of the tourniquet is not pathognomonic of purpura. It occurs in a great many other conditions. There is a difference in the results of this test in the use of an ordinary rubber tourniquet and the broad cuff of a sphygmomanometer. The latter produces positive results in a greater number of diseases than the former. Hyperthyroidism, hypertension, chronic nephritis, pernicious anemia, subacute bacterial endocarditis, and leucemia uniformly show positive reactions by the use of the sphygmomanometer. In some of these cases the test may be negative by the use of the ordinary rubber tourniquet. By the use of the rubber tourniquet, practically all the cases of purpura show a positive reaction.

The relation of the blood platelets to the capillaries is very important. Frank believes that the platelets prevent the diapedesis of red blood cells in the normal individual by stopping the various stomata between the capillaries which occur from the traumas of daily life. The diminution of the number of blood platelets in purpura interferes with this function because of the additional qualitative change. In other diseases where the blood platelets may be just as low as in thrombocytopenic purpura, purpuric manifestations are not very common as one can see from the number of cases reported in the various tables, a great many cases of pernicious anemia and subacute endocarditis with marked diminution of the blood platelets have not been included in this communication on account of the absence of purpura. Leschke and others believe that there is some alteration of the capillaries in thrombocytopenic purpura. In a few cases of purpura which we have followed, purpuric skin manifestations rarely occur but are easily produced by slight traumas. The so-called spontaneous purpuric manifestations are probably due to unnoticed or very slight traumatic changes of the capillaries. Even changes in venous blood pressure may bring on attacks of apparently spontaneous purpura, as one notices frequently in the legs (orthostatic purpura). The latter form of purpura may also occur in less well-known diseases of the capillaries which show increased fragility without any evidence of changes in the blood platelets (Arnett and Weidman³⁶).

4 Clot Retraction Hayem was the first one to point out the absence of clot retraction in cases of purpura during the active stage of the disease. His coworkers have also found this to be present in symptomatic purpuras where a diminution of blood platelets is also found. The experiments of

TABLE VII
ACUTE AND CHRONIC APLASTIC ANEMIA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	M M	33	11	1	1	53	58	5	15	24	+	None
2	RA	15	8	1	1	19	68	5	14	20	+	None
3	JS	24	7	1	1	10	89	1	15	22	+	None
4	M L	42	32	2	2	45	49	4	9	29	+	Slight
5	AS	40	17	1	30	22	72	5	6	8	+	Slight
6	AS	51	55	3	2	32	78	10	7	6	+	None
7	J T	25	14	2	5	29	63	7	16	19	+	None
8	A W	44	15	2	20	32	64	2	6	4	+	Slight
9	H O	29	15	1	10	44	46	7	17	13	+	None
10	J G	49	24	3	10	40	42	2	8	8	+	None

Cases 1-5 Acute Aplastic Anemia
Cases 6-10 Chronic Aplastic Anemia

Le Sourd and Pagniez³⁷ have definitely proved the relation of the blood platelets to clot retraction. Arthur and Chapiro³⁸ have shown that the clot retraction depends upon the integrity of the blood platelets. Sodium fluoride distilled water, and cold which injure the blood platelets retard retraction. Sodium citrate and sodium oxalate which conserve blood platelets do not hinder clot retraction. It is exceptional to find clot retraction in cases of purpura hemorrhagica; this was observed only in four cases where the blood platelets varied from 5,000 to 48,000.

There can also be a dissociation of function of the blood platelets. (1) The blood platelets may retain the adhesive properties and retractive properties, and cause the purpuric phenomena solely through their diminution in number. (2) The blood platelets may possess the adhesive properties but may be deficient in the retractive property. (3) The platelets may show both quantitative and qualitative changes.

The blood studies confirm Havem's idea⁴ that the two most important blood changes in true purpura are the diminution of the blood platelets and the loss of clot retraction.

3 *Chronic Aplastic Anemia (Table VII)*—Chronic aplastic anemia is separated from the chronic purpuric cases on account of the difference in their course and the blood picture. They resemble pernicious anemia on the one hand, except that there is an absence of macrocytosis and thrombocytopenic purpura on the other hand, except that the white blood cells are persistently low, with a relative lymphocytosis. We have had occasion to study five cases. Splenectomy was tried unsuccessfully in three; two died within a month after the operation and one patient is still living but has a very severe anemia. They all had attacks of purpura with the characteristic alterations in functions of the blood platelets. In one case macrophages were present and a positive blood culture of *Streptococcus anhemolyticus* was later reported.

4 *Acute Aplastic Anemia (Table VII)*—These cases were of short duration. They were all characterized by the presence of marked hemorrhagic phenomena. In Case 4 the bleeding had to be stopped at the end of twenty minutes by compression of the ear with gauze. Twenty-four hours later, when the gauze was removed, bleeding began again. The blood picture is charac-

terized by a marked diminution of all the formed elements of the blood, and a relative lymphocytosis. The bleeding time, capillary resistance test, and clot retraction were the same as in thrombocytopenic purpura.

B. SYMPTOMATIC THROMBOCYTOPENIC PURPURA

Certain diseases such as leucemia, subacute endocarditis, and pernicious anemia may be associated with purpura. As a rule the underlying condition can be determined by clinical and laboratory examinations. The true nature of the purpura cannot be determined without the complete blood picture.

1 *Leucemia (Tables VIII, IX, and X)*—Leucemias with a normal or diminished white cell count simulate thrombocytopenic purpura very closely. The symptoms may be identical. The only means of differentiating the two conditions is the blood picture. As a rule the anemia is out of proportion to the amount of blood lost, but otherwise the platelets and the special tests

TABLE VIII
LEUCOPENIC MYELOID LEUCEMIA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	MCN	MBST	CT	BT	TT	CR
1	HP	44	2.0	1	38	18	61	6	1	14				
2	JG	50	2.6	2	6	6	52	2	4	35	5	20	+	None
3	RS	24	1.5	2	2	16	37			47	12	24	+	None
4*	ST	42	2.2	3	40	27	29	44			10	8	+	None
5	IR	27	1.1	3	36	13	65	1	1	19	7	3	+	None
6	HZ	30	1.0	3	60	42	40	8	4	47	10	4	+	Slight
7	EB	18	1.4	5	60	40	41	8	7	3	6	3	+	Normal
8	JH	80	4.0	5	9	18	65		4	13	12	24	+	None
9	MW	58	2.7	5	10	17	71	2	2	8	14	15	+	None
10	AW	14	8	8	20	44	18		16	22				
11	SS	30	1.4	9	35	61	11	1	8	19	10	16	-	Slight
12	SR	38	1.9	9	6	50	39	4	4	3	7	4	+	Slight
13	MC	44	2.4	11	32	5	8			87	5	20	+	None
14†	SW	52	2.2	12	30	68	18	1	10	3	4	5	-	None
15	TF	30	1.6	13	96	68	4	2	6	20	10	2	+	Normal
16	DS	30	1.6	13	20	31	45	2	8	11	12	6	+	None
17	LD	69	4.4	18	40	4	25	4	5	63	24	1	-	None
18	BW	56	2.9	20	4	26	27		1	70	7	3	+	Slight

*Monocytic leucemia

†Splenectomy—death

MCN—Myelocytes Neutrophilic

MBST—Myeloblasts

TABLE IX
MYELOID LEUCEMIA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	MCN	MBST	CT	BT	TT	CR
1	MC	20	1.3	28	15	13				87	4	3	+	Normal
2	HW	19	1.0	30		1	1	2	10	85	12	120	+	None
3	BM	42	1.5	32	20	4	10	1		85	4	30	+	Normal
4	BB	33	1.6	47	10	2	14			85	8	20	+	None
5	AG	13	7	53	20	5	1	1	1	88	4	20	+	None
6	AH	40	1.8	63	30	5				95	7	13	+	None
7	AP	58	2.8	71	50		2			97	10	15	+	None
8	GF	62	3.8	100	50	41	40	6	7	5	14	15	+	None
9	CI	50	2.9	124	15	20	26	14	9	21	5	6	+	None
10	BM	30	2.6	135	15	20	10			54	15	25	+	None
11	DG	93	4.6	141	60	2	20			78	11	30	+	None
12	IK	32	1.5	200	55	20	8		2	88	3	12	+	Slight
13	MK	30	1.2	600	20				1	97	10	8	-	None

MCN—Myelocytes Neutrophilic

MBST—Myeloblasts

TABLE V
LYMPHATIC LEUCEMIA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	LBST	CT	BT	TT	CR.
1	IS	30	3	5	85	1	99			2	14	+	Normal
2	IF	32	17	7	12	1	57	1	40	8	10	+	None
3	HC	120	59	8	120	26	74			9	5	+	Slight
4	SF	80	43	8	50	20	77	3		10	4	+	Slight
5	HE	52	41	17	60	17	83	1		10	8	-	Normal
6	JS	101	53	23	1	13	83			12	20	+	None
7	LB	83	51	33	2	1	91			4	1	-	Normal
8	AM	49	15	41	10	2	16		81	14	1	+	None
9	TB	35	18	42	6	-	64	4		11	120	+	None
10	SG	45	22	69	10	-	98			4	10	+	Slight

LBST—Lymphoblasts

TABLE VI
SUBACUTE BACTERIAL ENDOCARDITIS WITH PURPURA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	MAC	CT	BT	TT	CR.
1	AS	59	37	13	8	95	3	1		11	6	+	None
2	MD	54	11	-	18	()	4			9		+	None
3	BC	70	14	4	3	59	1	10		8		+	Slight
4	AF	67	36	-	0	63	26	8	1	8	4	+	None
5	EE	74	56	-	70	10	28	2		7	2	-	Slight
6	RG	49	21	-	10	1	27	6		9	3	-	Normal
7	HK	71	47	42	-	6	20	4	10	12	10	+	Slight
8	DV	58	27	-	150	62	31	6		4	2	+	Normal
9	JG	70	41	-	130	27	0	6	12	4	3	+	Normal
10	AF	71	47	-	160	9	18	3		6	9	+	None
11	SE	50	33	4	100	60	26	-			1	-	Normal

MAC—Macrophages

correspond to those found in thrombocytopenic purpura. The important differential point is the presence of premature cells, such as neutrophilic myelocytes, myeloblasts and their forerunners, the myelocytes in the leucopenic myeloid leucemias, and the lymphocytosis and lymphoblasts in the leucopenic lymphatic leucemias. The differential blood count is therefore extremely important in all cases of purpura. The staining methods and the differentiation of the various premature white blood cells have been discussed in a previous communication. The leucocytic leucemias offer no difficulty in diagnosis. The high white cell count is diagnostic. It is interesting to note that most of the leucemias which are associated with purpura are of the myeloblastic type.

2 *Subacute Bacterial Endocarditis* (Table VI).—In a large series of cases (over 100) studied as outlined above the blood picture was quite varied. The secondary anemia was progressive in practically all of the cases. The leucocytes varied from a marked leucopenia (usually in the bacterial free stage with splenomegaly) to leucocytosis. The platelets also show the same variations. The differential blood picture showed marked changes, polymnucleosis in some lymphocytosis in others, and in about 10 per cent of the cases, macrophages or histiocytes were found in the blood smear from the ear. Skin manifestations were present in practically all, consisting of bright red petechiae, white centered petechiae, and in about twenty patients purpura was present. The blood of eleven of these patients was carefully studied. In five, macrophages were present at one time or another. In most of the cases the platelets were very low and in two cases the platelets were 160,000 and 200,000 with normal bleeding time in both. The tourniquet test was positive in eight of

TABLE XII

SPLENOMEGALY AND PURPURA SHOWING TENDENCY OF DIMINUTION OF WHITE CELLS AND PLATELETS, RESEMBLING CASES OF BANTI'S DISEASE

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	M H	28	1,6	2	0	83	15	2	8	7	+	None
2	F S	52	3,3	5	10	64	27	8	10	6	+	None
3	A I	20	1,6	3	15	57	40	3	6	9	+	Slight
4	E B	32	2,4	2	20	73	18	8	8	9	+	None
5	J S	100	4,4	3	40	54	38	6	10	4	-	Slight
6	E L	46	2,3	3	60	39	60	1	12	8	-	None
7	J D	26	1,5	4	70	80	16	4	5	2	-	Normal
8	A S	88	4,9	9	145	58	38	4	8	3	+	Normal

TABLE XIII

GAUCHER'S DISEASE

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	F R	55	3,1	3	30	47	50	3	5	9	+	Normal
2	H L	57	2,8	1	32	67	23	6	15	6	+	None
3	L T	53	3,3	4	50	57	39	4	7	7	+	Slight
4	I R	90	4,6	5	60	73	22	4	10	2	+	Slight
5	S P	90	5,0	2	60	74	21	4	10	6	+	Slight
6	S F	60	3,6	2	90	46	52	2	11	5	+	Slight
7	M C	45	3,1	2	110	52	38	8	7	3	+	Normal

the eleven cases. In three cases there was an absence of clot retraction. In the others the clot retraction was either normal or slight. The purpuric changes present in subacute bacterial endocarditis can be said to be due to some capillary change as well as some alteration of the function of the blood platelets. The latter is not as pronounced as it is in true cases of purpura. Clinical phenomena are more important in the diagnosis of this disease, although the finding of macrophages in certain obscure cases without a positive blood culture makes the diagnosis of this condition certain.

3 *Splenomegaly* —

(a) In eight patients with splenomegaly whose blood pictures resembled that found in Banti's disease, purpuric manifestations were very marked and, except for the leucopenia, may be classified as thrombocytopenic purpura (Cases 1, 2, 3, 4, and 7). All these patients reacted favorably after splenectomy. In the other three cases, purpura was very slight and transitory, and did not play an important rôle. Case 5 was Banti's disease in the third stage, splenectomy was followed by death. The relation of the blood platelets to Banti's disease is a very interesting one and has been reported previously (Rosenthal³⁹) (Table XII).

(b) Gaucher's Disease (Table XIII). Seven patients with Gaucher's disease showed the presence of a typical purpura, the characteristic diminution of the blood platelets, slightly increased bleeding time, positive tourniquet test, and normal clot retraction were found in two cases, and absence of the clot retraction in the others. The diagnosis of this condition cannot depend upon the blood examination alone. The clinical manifestations of the disease, enlargement of both liver and spleen, pigmentation, pingueculae, and sometimes gross or x-ray bone changes are necessary for the diagnosis. When the liver and spleen alone are enlarged, without the other changes, bone or splenic puncture may be required for the diagnosis of the condition.

TABLE XIV
PERNIOUS ANEMIA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR.
1	SI	33	1.4	2	20	51	46	3	9	8	-	Slight
2	MM	40	1.5	5	50	54	41	2	9	8	-	Normal
3	MR	76	3.4	4	80	37	61	2	10	3	+	Normal
4	AS	44	1.4	4	80	58	30	3	9	5	+	Slight
5	MI	34	1.1	4	90	59	34	3	8	7	+	Slight

TABLE XV
TUBERCULOSIS

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR.
1	MC	90	4.7	6	2	68	27	6	12	17	+	None
2	PL	33	2.7	12	25	72	19	8	5	16	+	None
3	JS	64	4.0	14	64	31	45	14	10	8	+	None
4	DO	40	2.6	9	80	19	17	4	12	19	+	None

(c) *Acquired Hemolytic Icterus* —Purpura was found in one case of acquired hemolytic icterus. The blood platelets were diminished to 80,000, but these were apparently of good quality as the bleeding time and clot retraction were normal and the tourniquet test was negative. Bile in the blood and increased fragility of the red cells were present. Hemoglobin, 32 per cent, red blood cells, 2,600,000, white blood cells 3,000, platelets 80,000, polymorphonuclear neutrophils, 76 per cent, lymphocytes 20 per cent, monocytes, 4 per cent, coagulation time four minutes, bleeding time three minutes, tourniquet test negative, clot retraction normal.

4 *Pernicious Anemia* (Table XIV) —The blood picture is the most important means of making the diagnosis of pernicious anemia. The high color index and the macrocytosis is rarely found in other conditions except occasionally in leucopenic leucemia. The differential blood count in pernicious anemia shows a slight lymphocytosis and rarely the presence of premature blood cells. Normoblasts and megaloblasts are rather infrequent in pernicious anemia, and, as a rule, are present in very large numbers in the leucopenic leucemias. Most of the cases showed a marked diminution in the number of blood platelets. Purpura is not common and was found in only five cases. The qualitative change in the blood platelets is not as great as it is in purpura.

5 *Tuberculosis* (Table XV) —Purpura is rather uncommon in tuberculosis. In two of the cases the purpuric manifestations were the outstanding features. The blood picture was typical of thrombocytopenic purpura in both. Further observations of the cases disclosed the presence of tuberculosis. Case 1 (Table XV) revealed the presence of tuberculosis in an excised lymph node, and the x-ray examination of the chest showed the presence of milary tuberculosis. In Case 2 tuberculosis was found in one kidney, which was excised. The purpura disappeared in both kidneys, and the blood picture in Case 2 became normal, the blood platelets increased in number, and the special tests for their quality also showed normal results. Case 3 was a child who showed clinically a severe anemia, marked enlargement of the spleen, and purpura. Examination of the spleen after splenectomy showed microscopic tuberculosis. For three years after the operation the child showed a blood picture which resembled

bled monocytic leucemia and absence of a hemorrhagic tendency Case 4 showed a terminal purpura in general military tuberculosis

6 *Purpura as a Result of the Intake of Drugs*—It is usually stated that numerous drugs may produce purpura Skin manifestations nonpurpuric in character are frequent as a result of the intake of various drugs True purpura as a result of drugs was found in only two cases One patient had an idiosyncrasy for quinine and would always develop a purpuric eruption following its ingestion The blood was examined during one attack and was found that the blood platelets were markedly diminished, coagulation time was normal, bleeding time was normal, tourniquet test became strongly positive, and no clot retraction was present The purpura disappeared within a week, and the idiosyncrasy was tested by injecting a minute quantity of urea and quinine hydrochloride intradermally As a result he developed a profuse purpura scattered all over the body Hemoglobin, 90 per cent, red blood cells, 5,000,000, white blood cells, 16,000, platelets, 90,000, polymorphonuclear neutrophils, 50 per cent, lymphocytes, 42 per cent, monocytes, 6 per cent, coagulation time, nine minutes, bleeding time, three minutes, tourniquet test, positive, clot retraction, none

Purpura after salsolan was observed in one case, although this was stated to be more frequently found in France where the subject has been thoroughly investigated (Mattas⁴⁰) In the case under observation, the purpuric manifestations cleared up after three citrate transfusions, with the restoration of a normal blood picture The blood picture in this case showed typical findings of thrombocytopenic purpura Hemoglobin, 42 per cent, red blood cells, 2,900,000, white blood cells, 14,000, polymorphonuclear neutrophils, 83 per cent, lymphocytes, 13 per cent, monocytes, 3 per cent, coagulation time, ten minutes, bleeding time, fifty-five minutes, tourniquet test, positive, clot retraction, none

7 *Carcinoma*—Both cases showed a typical blood picture of thrombocytopenic purpura Case 1 showed widespread metastases of the long bones and in the other case, no evidence of metastasis of the long bones could be found Purpura was found in one patient who had a dermoid cyst of the ovary After the removal of the tumor, the purpura cleared up The relation of purpura to the ovarian function is discussed by Meyerstein⁴¹

8 *Typhoid Fever*—The blood picture in this case is that of the thrombocytopenic variety The patient showed a very marked hemorrhagic tendency with the development of the purpura, and died from exsanguinating intestinal hemorrhages which numerous transfusions failed to stop

C CHRONIC THROMBASTHENIC PURPURA

Glanzmann⁴² differentiates this condition from hemophilia by the presence of a prolonged bleeding time and absence of clot retraction Van der Zande⁴³ observed a family in which thrombasthenia was present both in male and female members The clot retraction was present but somewhat retarded The two cases here reported occurred in a mother (Case 1) and a son (Case 2) They are mild bleeders and have had attacks of epistaxis, purpura, and ecchymoses It is interesting that an abdominal operation in one case was

not followed by undue hemorrhage. The coagulation and bleeding times were distinctly prolonged in both. Case 1 hemoglobin, 95 per cent red blood cells, 6,000,000, white blood cells 9,000 platelets, 180,000, polymorphonuclear neutrophils, 50 per cent lymphocytes 42 per cent monocytes, 6 per cent, coagulation time, twenty six minutes bleeding time, six minutes, tourniquet test, negative, clot retraction, normal. Case 2 hemoglobin, 90 per cent, red blood cells, 5,400,000 white blood cells, 13,000 platelets, 240,000, polymorphonuclear neutrophils 59 per cent lymphocytes, 36 per cent, monocytes, 5 per cent, coagulation time sixteen minutes bleeding time, ten minutes, tourniquet test, negative, clot retraction normal.

D. HYPERTENSION AND NITROGEN RETENTION (TABLE XVI)

Anemia is always present in such cases as reported by Beig⁴ and others. The occurrence of purpura is rather unusual in such cases although other forms of hemorrhages (burn retina nose stomach and intestines) are not unusual. The platelets are normal or even increased and the clotting time is prolonged in most of the cases.

In the six cases with purpura which came under observation five showed a diminution of the blood platelets (50,000-110,000) a moderately increased bleeding time, and normal clot retraction. The tourniquet test was positive in all. Case 6 showed very marked arteriosclerotic changes which were partly responsible for the hemorrhagic condition (so called arteriosclerotic purpura).

E. JAUNDICE (TABLE XVII)

The bleeding tendency in certain cases of jaundice is attributed to some fault in the coagulation of the blood as a result of some change in the coagulation factor (Morawitz, Lee and Vincent). Purpura is not a common symptom and was noticed in only four cases. The platelets do not seem to play an important role in its production. In Case 4 there was a marked prolongation of the bleeding time and coagulation time. The tourniquet test was positive in all cases. The condition of the capillaries possibly is also affected by the jaundice and may be partly responsible for the prolonged operative bleeding time (Leschke) in addition to the altered coagulability of the blood.

TABLE XVI
HYPERTENSION AND NITROGEN RETENTION

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	HG	70	4.2	10	50	70	27	4	10	5	+	Normal
2	LD	54	2.1	5	60	68	29	0	10	10	+	Slight
3	IB	55	2.0	, 10	64	31	4	8	2	+	Normal	
4	IM	42	2.1	4	10	70	22	3	15	3	+	Normal
5	DW	54	2.5	16	110	91	1	1	10	15	+	Normal
6	JR	70	3.6	12	190	68	27	0	16	6	+	Normal

TABLE XVII
JAUNDICE

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CP
1	B K	55	2.7	7	126	80	19	1	30	3	+	Normal
2	H T	90	4.7	8	175	83	12	4	5	1	+	Slight
3	S S	92	6.0	8	260	78	21	1	12	5	+	Normal
4	A N	65	3.8	12	310	80	14	6	24	12	+	Normal

TABLE XVIII

SCURVY

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	ET	40	2,2	4	220	67	30	3	7	3	+	Normal
2	AM	75	3,9	7	290	74	22	4	12	1	+	Normal
3	EW	58	2,7	4	480	68	29	2	4	8	+	Normal

TABLE XIX

SCHOENLEIN HENOCH'S PURPURA

NO	NAME	HGB	RBC	WBC	PL	PN	L	M	CT	BT	TT	CR
1	HP	100	5,2	11	190	53	36		7	2	+	Normal
2	PG	80	4,0	10	200	7,0	20	5	5	5	+	Normal
3	ML	94	4,8	6	210	56	42	2	9	2	+	Normal
4	SS	80	3,8	7	230	57	36	6	7	3	+	Normal
5	CM	98	4,8	5	240	66	22	5	12	3	+	Normal
6	JB	80	4,4	8	240	42	55	3	9	3	-	Normal
7	MD	88	4,5	8	410	64	27	4	12	1	-	Normal
8	FR	48	3,3	21	420	87	7	6	6	3	+	Normal
9	SP	90	4,6	17	610	87	10	2	12	2	-	Normal

F SCURVY (TABLE XVIII)

Except for the positive tourniquet test, the blood picture, in cases of scurvy, is normal. Anemia may occur after excessive hemorrhages of long duration. The diagnosis of this condition depends upon the history of the deprivation of the individual from foods containing vitamin C.

G SCHOENLEIN-HENOCH'S PURPURA OR PELLIOSIS RHEUMATICA (TABLE XIX)

Schoenlein (1839) described a form of purpura associated with a joint involvement, and in 1868 Henoch described similar cases with colic and intestinal hemorrhages. The purpuric manifestations are sometimes associated with erythema and urticaria which rarely occur with thrombocytopenic purpura. The etiology of this condition is still unknown. Frank⁴⁵ believes that it is due to some process of autointoxication, probably from split protein products resembling histamine, producing a toxic effect on the capillaries. This disease has also been attributed to tuberculosis. Castex,⁴⁶ in the study of two cases, noticed the symmetrical distribution of the purpura and found definite changes in the sympathetic nuclei.

The blood picture is normal except for the positive tourniquet test. The blood platelets are not altered either quantitatively or qualitatively, and only offer confusion with the true thrombocytopenic purpuras when the blood examination is omitted.

H PURPURA FULMINANS

Very little is known about the cases of purpura fulminans, which usually occur in children. The blood picture reported in our case was in an adult. The condition may be a fatal form of the Schoenlein-Henoch's purpura group. Hemoglobin, 84 per cent, red blood cells, 5,200,000, white blood cells, 19,000, platelets, 250,000, polymorphonuclear neutrophils, 86 per cent, lymphocytes, 10 per cent, monocytes, 4 per cent, coagulation time, seven minutes, bleeding time, two minutes, tourniquet test, positive, clot retraction, normal.

SUMMARY

An analysis of the blood picture of the cases studied shows that purpura may be divided into three main groups (1) Purpura characterized by a diminution of the blood platelets with or without alteration of their functions, (2) purpura due to some change in the function of the platelets without a diminution of their number, (3) purpura due to changes in capillaries

1 The blood picture in the first is diagnostic in cases of acute and chronic thrombocytopenic purpura. These are characterized by diminution of the blood platelets with or without alteration of their function. Anemia is present after hemorrhages only. The number of blood platelets does not seem to bear any definite relation to the bleeding time, but occasionally this property may not be altered. Coagulation time is normal or slightly prolonged. The tourniquet test is exceptionally absent. The clot retraction is always impaired except in rare instances. The diagnosis of this condition can be definitely made from the blood picture when no cause is found to account for the hemorrhagic tendency. This condition is an acute or chronic constitutional disturbance, as a result of either altered blood platelet formation or increased blood platelet destruction.

The blood platelets are also diminished in acute and chronic aplastic anemias, anemia and leucopenia are also present. The anemia is far greater than can be accounted for by the hemorrhages. The diminution of blood platelets is always associated with qualitative changes.

The diminution of blood platelets may be associated with other diseases (symptomatic thrombocytopenic purpura). The blood picture is diagnostic in the leucemias, frequently in subacute bacterial endocarditis, when macrophages are present, and in pernicious anemia. Attention again must be called to the fact that some cases of subacute leucopenic myeloblastic leucemia may be confused with thrombocytopenic purpura unless a careful differential blood examination is done for the identification of the premature myeloid cells, such as myeloblasts and myelocytes. The splenomegalic conditions associated with purpura require other examinations for their identification. Cases of tuberculosis, carcinoma, subacute endocarditis, drug poisonings, and typhoid fever closely resemble thrombocytopenic purpura, but require special clinical observations for their identification. As a rule the purpura is not the outstanding feature in such cases.

2 The second group, chronic hereditary thrombasthenic purpura, is rare, and still requires further study before it can be definitely said that the blood platelets are wholly responsible for the condition. All the cases so far reported seem to be inconstant in the blood findings with the exception of the normal platelet count.

3 The third main group is purpura due to conditions which affect the capillaries. Schultz⁴⁷ classifies all the conditions as athrombopenic purpura, but it is far outnumbered by the thrombocytopenic purpuras. The various conditions which we have found that may affect capillaries are jaundice, hypertension associated with nitrogen retention, vitamin deficiencies, and Schoenlein-Henoch's purpura the cause of which is not known. Most of them

are characterized by no change in the number of blood platelets, or by qualitative changes

In hypertension and nitrogen retention the blood platelets may also be diminished. Subacute bacterial endocarditis also shows evidence of marked capillary disturbances as shown by the frequent appearance of petechiae and a positive capillary resistance test. Case 4 (Table XVI) showed no blood diminution of the blood platelets, but presented marked purpuric manifestations.

CONCLUSIONS

1 The ordinary blood examination is insufficient for the differentiation of the various types of purpura. In addition the blood platelets must be counted and their functions studied in each individual case.

2 The blood picture shows three main groups of purpura.

(a) Thrombocytopenic purpura. Purpura is either the predominating symptom as a result of a diminution of the blood platelets, or it may be symptomatic.

(b) Chronic thrombasthenic purpura (rare). The blood platelets are normal in number but show qualitative changes.

(c) Purpura as a result of alteration of the capillaries due to numerous causes. In a few cases there may be an additional disturbance of the blood platelets, as in uremia and subacute endocarditis.

3 A classification is presented as a result of a systematic study of 172 cases showing purpura as a primary or secondary symptom.

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DISCUSSION

Dr Euben Ottenberg—With regard to the tourniquet test it seems to me that blood pressure has something to do with it. If a standard time and pressure are used the method is perfectly satisfactory as petechial spots do not occur in normal individuals. If the pressure is excessive petechial spots are obtained in all individuals. Leave the tourniquet on for three minutes at a pressure intermediate between the systolic and the diastolic blood pressure. Under those conditions normal persons do not show petechial spots and abnormal ones do.

Dr Reed Rockwood—What method did you use for the determination of blood platelets and what is its approximate error? Have you made any observations upon the morphology of the platelets in the thrombocytopenic form of purpura by means of the ultramicroscope? A very few observations which I made in this group of cases show that there is a distortion of the structure of the platelets which is made visible by this method of study.

Dr Rosenthal (closing)—The time allowed did not permit me to take up the counting of the blood platelets and their morphology. As to their enumeration we have used two methods. The first is the use of the 3 per cent sodium citrate similar to that of counting

II are given the results of blood counts done at approximately the same time as that at which the sedimentation test was carried out, and the clinical diagnoses on the abnormal subjects studied. If the figures for the red cell counts are compared with the results in Table I, it is plain that in some instances rates which would be at least classed as suspicious were obtained upon subjects with normal sedimentation rates when the number of red cells was reduced to figures which would not indicate a very severe grade of anemia. Case 5, when an artificially produced anemia of about 3,700,000 cells per cmm was present, showed a sedimentation time of sixty-one minutes, which is usually accepted as distinctly abnormal by this method, and Case 7, which had a sedimentation time of 261 minutes when the red blood count was 5,450,000 per cmm, gave a value of only ninety-one minutes in the specimen which corresponded with a count of approximately 3,940,000 per cmm. A similar marked change can be demonstrated when the experiments made the blood more concentrated. For example, Case 10 showed a settling time of seventy-eight minutes a value which has not infrequently been considered

TABLE I
SEDIMENTATION TIME (IN MINUTES)

NUMBER		5 CC BLOOD PLUS PLASMA							5 CC BLOOD MINUS PLASMA			
CASE	SERIES	00 cc	05 cc	10 cc	15 cc	20 cc	25 cc	50 cc	05 cc	10 cc	15 cc	20 cc
1	14	442	281	225	174	144	—	—	—	—	—	—
2	13	274	160	140	112	101	—	—	—	—	—	—
3	11	233	—	193	—	—	87	61	—	—	—	—
4	10	496	358	230	148	115	—	—	—	—	—	—
5	12	117	100	78	—	61	—	—	—	—	—	—
6	16	232	182	143	124	111	—	—	—	—	—	—
7	5	261	—	152	—	91	—	—	—	—	—	—
8	6	58	—	37	—	27	—	—	—	133	—	—
9	3	95	—	—	—	—	—	—	—	530	1961	—
10	2	78	—	—	—	—	—	—	102	120	—	415
11	8	43	—	—	—	—	—	—	54	59	69	76
12	4	45	—	—	—	—	—	—	55	76	—	520
13	7	7	—	—	—	—	—	—	12	17	—	21

TABLE II

CASE	SERIES	SEX	BLOOD COUNT				DIAGNOSIS
			ERYTH ROCYTES NO	LEUCO CYTES NO	HEMO GLOBIN %	NEUTRO PHILES %	
1	14	M	—	—	—	—	Normal
2	13	M	5,500,000	6,400	87	50.2	Normal
3	11	M	5,350,000	7,400	85	48.0	Normal
4	10	M	5,180,000	10,200	97	75.0	Normal
5	12	M	5,030,000	8,000	93	45.1	Normal
6	16	F	4,400,000	8,800	79	65.0	Normal
7	5	M	5,460,000	23,600	106	22.2	Diabetes, furunculosis
8	6	M	3,950,000	11,800	80	70.4	Sprue
9	3	F	3,930,000	7,000	71	49.0	Acute trigonitis
10	2	M	3,700,000	9,800	68	67.3	Pernicious anemia
11	8	M	2,850,000	25,600	29	91.0	Carcinoma of stomach, secondary anemia, Glycosuria
12	4	F	4,050,000	8,000	71	62.7	Sarcoma, metastatic, axillary glands and bladder
13	7	M	3,250,000	15,600	47	75.7	Abscess of lung

abnormal when the blood count was 3,700,000 per c mm. When the specimen was concentrated to approximately 5,180,000 cells, the time was 120 minutes, or approximately normal.

It does not seem best to place too great emphasis upon such calculations for several reasons. In the first place it cannot be said with certainty that such dilutions and concentrations correspond to the changes that may take place in the body in anemia although it seems to us that this plan serves as a method for studying the effect of such changes by themselves better than any other experimental technique which can be devised. In the second place such calculations place all of the emphasis upon changes in the number of cells, while it is not impossible that the variations in sedimentation time correspond with changes in cell volume rather than cell concentration. The number of cells is the most important factor which determines cell volume but it is not the only one upon which it depends; the average size of the cell is also a factor. A third reason for not laying too much stress on the results is that changes in cell volume or of cell concentration may apply only to the methods used for measuring the sedimentation time. It is almost tacitly assumed, it seems to us, that the rate at which the column of red cells settles out from solution is to some extent a measure of the rate at which the average red cell would settle. From our experiments it seems almost certain that this is not entirely true. A fact that may apply only to a method of measuring a phenomenon may have little or no physiologic significance. On the other hand changes in cell volume, or cell concentration almost certainly does affect any of the methods of estimating the sedimentation time now in use, as has already been shown by Rubin for one of the other standard techniques and therefore must be taken into account when such readings are interpreted.

There are a number of possible plans for correcting the "error" produced by variations in cell concentration. One is that discussed by Rubin and Smith⁷ who have suggested that results be expressed as the ratio between the amount of sedimentation in one and twenty four hours. This is approximately although not exactly an expression of the ratio between sedimentation rate and cell volume. Another method would be to determine an average "normal" sedimentation rate to correspond with different cell concentrations, hemoglobin contents, and cell volumes. This is obviously impossible because the differences among normal subjects with approximately the same blood picture is so marked, a point which has been repeatedly brought out in the literature and which is clearly shown by the tables. Another thing which a study of Table I reveals is that there is no constant quantitative relationship between the percentage of changes produced in cell concentration (or cell volume) and the sedimentation rate. It would not be possible to determine the sedimentation time and blood count, or cell volume on a given patient, and calculate what the rate would be for a different blood count. A number of other plans for working out the effect of cell concentration in a given case were tried, but none of them can be described as wholly satisfactory. Our technique which was carried through several times was as follows: the cell volume was determined on the blood as drawn and on each specimen which had been diluted with plasma by centrifuging at 2500 revolutions per minute

for thirty minutes, cells from each of the diluted specimens were then mixed with plasma in the same proportions in which they were found in the initial blood, and sedimentation times determined. On the average the results agreed very well with those obtained on the initial blood specimen, but in several instances such marked discrepancies were found that the method cannot be recommended. The sources of error include mistakes in technic (the high viscosity of the cells makes accurate measurement very difficult), a possible effect of standing, which was probably slight, and electrical changes induced by the excessive manipulation. In our opinion this last is the most serious source of error. A better plan would be to allow a measured amount of blood to stand until the cells have partially settled and then remove a portion of the plasma calculated to produce the desired cell concentration. Such a technic would avoid the more important sources of error found when cells and plasma were separated, and would be liable only to that produced by standing—an error which our own results and those of others seem to show is relatively small. It must be borne in mind, of course, that such a correction may not bear any relationship to conditions in the body.

There is a distinct relationship between the rate of sedimentation of erythrocytes on the one hand and their concentration in the blood on the other. Rather small variations from normal blood counts may apparently produce quite marked differences in the sedimentation rate as usually measured. When the sedimentation time is used clinically, this fact should be kept in mind, for other factors cause marked deviations from the normal, and it is in these other factors that the clinician is primarily interested in his application of the test.

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STUDIES OF SEDIMENTATION OF ERYTHROCYTES*

By HENRY F. HUNT † M.D., ROCHESTER, MINN.

TECHNIC

SOME of the most ancient writers in medical literature refer to the difference in the sedimentation time of blood cells in specimens of drawn blood. Galen noticed, particularly in acute inflammatory processes, that the cells became separated from the plasma even before coagulation had set in. Later John Hunter, Virchow and others made the same observations. The clear yellowish serum that was left by the sinking away of the red cells was called the "crusta inflammatoria" or "crusta phlogistica," and was regarded as an exceedingly important sign. The color, thickness and consistency of the crusta inflammatoria were considered significant in the diagnosis, treatment, and prognosis of the disease. As the practice of bleeding patients fell into disrepute, the sedimentation of the blood cells and the crusta inflammatoria were forgotten.

Biernacki, in 1897 reported a series of seventy-five sedimentation tests. He concluded that the test might be of some value in the diagnosis of diseases. Robin Fahraeus, in 1917, rediscovered this phenomenon and his observations were published in articles in *Hygiea* in 1918 and in the *Biochemische Zeitschrift* of the same year. He believed the condition might be used as a test for pregnancy as there was a marked difference between the sedimentation of the blood cells in parturient and nonparturient women. He also called attention to the fact that in cases of infectious diseases and malignant tumors as well as during pregnancy there is acceleration of the sedimentation rate.

Fahraeus continued to work on this problem and published an article in which he advanced the theory that the increased sinking velocity of the red cells (or increased sedimentation) which has been shown to exist during pregnancy, and many diseases is dependent on either an increased agglutination of the corpuscles or on a decrease in their number. The agglutination here in question is identical with the rouleau formation of the red blood cells, and the increased agglutination is only an exaggeration of the normally existing phenomena. Fahraeus also noted that in the cases in which the agglutination was increased, there was a corresponding increase in either the serum globulin or the fibrinogen or both.

The etiologic factor in blood sedimentation has been extensively investigated. An increase or change in the electrical charge of the red cells has been held responsible by certain investigators. An increased cholesterol

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content, changes in viscosity of the blood plasma, variations in the erythrocyte count, and certain chemical changes in the blood have been described as causative factors, but none has been definitely proved

METHODS OF ERYTHROCYTE SEDIMENTATION

Many of the methods of carrying out blood sedimentation tests are essentially the same, but they differ in the amount of material used, end point, size of tube, time of reading, and collection of the specimens

Waugh using Linzenmeier's method collects 0.8 cc of blood in a special graduated syringe containing 0.2 cc of a 5 per cent sodium citrate solution. He then transfers this to a small sedimentation tube about 65 cm high and 0.5 mm clear diameter, with calibration marks at 10 cc, and at distances of 6, 12, 18, and 24 mm, respectively, from the 10 cc mark. The sedimentation tube is filled exactly to the 1 cc mark with the citrated blood, and the line of separation between the blood corpuscles and the plasma is noted as it reaches the various marks, the time that this takes is reported as the sedimentation time.

The Westergren and Fahraeus technique, as described by Friedlaender, differs considerably from the Linzenmeier method. With a syringe holding 0.4 cc of a 2.5 per cent sodium citrate solution, blood is drawn from a vein to the 2 cc mark. The blood and citrate are well mixed, and then transferred to a 1 cc pipette up to the zero mark. After one hour the column of erythrocytes is marked at its line of separation from the plasma and is measured in millimeters. A plasma column of from 3 to 15 mm in one hour is considered normal. Westergren, in a recent article, describes a more improved technique, although the fundamental principles are the same.

Zeekwer and Goodell collect 8 cc of blood in a graduated centrifuge tube containing 2 cc of 3 per cent sodium citrate solution. The mixture is allowed to stand, and the readings of the heights of the red cells are made at the end of one hour.

Cooper has combined the methods of Fahraeus, Zeekwer, and Goodell. He draws the blood into a bottle into which 3 drops of 20 per cent oxalate has been allowed to dry. This anticoagulant is sufficient for from 15 to 20 cc of blood and is usually used in conjunction with the taking of a specimen of blood for chemical analysis. Into a dry 15 cc graduated test tube is placed 5 cc of blood. This is allowed to stand in a rack, and readings are made at the following periods: five, ten, fifteen, thirty, forty-five, and sixty minutes, to note the level to which the cells have settled. The blood is centrifugalized at high speed for ten minutes, and the reading which is taken corresponds to that of the cell volume. This reading is practically identical with that obtained by twenty-four hours' sedimentation.

The technique of the test as used by Rubin is as follows. Into a sterile 2 cc record syringe a solution of 3.8 per cent sodium citrate solution is drawn to the 0.4 cc mark. Blood is then aspirated from a vein in the arm to the 2 cc mark, giving a dilution of 1:4. After this has been thoroughly mixed, it is drawn into long serologic pipettes graduated to hundredths, placed on a rack, and the layer of clear plasma observed at the end of one, two, and twenty-

four hours, the readings noted directly in percentage of 1 cc. He considers the two hour reading as the most significant one.

After the literature had been carefully reviewed for different methods and many of them tried, I decided to modify the technique described by Westergren and Rubin. Since it was evident from preliminary work that it was unnecessary and inconvenient to use special syringes containing citrate or oxalate solutions and since it is difficult to obtain many tubes of uniform diameter, I used the ordinary 1 cc. serologic pipettes. The problem was to obtain a great many specimens in a short time, hence blood was aspirated from the median basilic vein using a dry 5 cc. syringe. Three and five

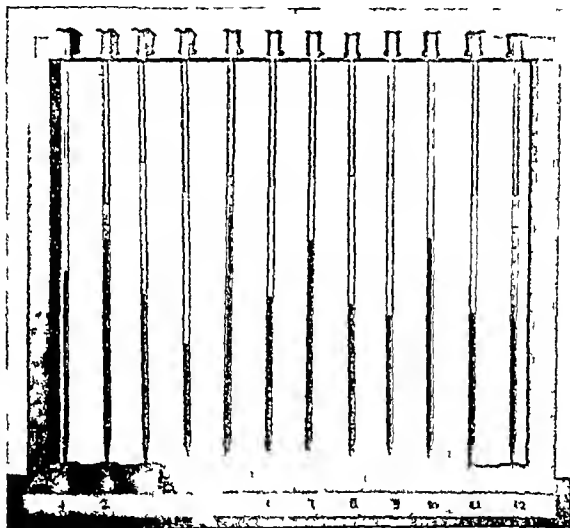


Fig 1—Set up for sedimentation test

tenths cubic centimeters of the blood was transferred to a graduated centrifuge tube containing 0.5 cc. of 16 per cent sodium citrate solution. The tube was then inverted several times so as to insure a thorough mixing of the blood with the solution. Exactly 1 cc. of the mixture was then drawn up into a 1 cc. serologic pipette graduated to hundredths placed in the special rack designed by Westergren and the separating line between the plasma and cells observed at the end of one two and twenty four hours (Fig 1). The reading obtained by using the graduated pipettes represents a definite percentage of the total volume of blood used.

There has been a great deal of confusion in the method of reporting the results of the sedimentation reaction. Some report the time that it takes the top of the column of red cells to reach a certain level others the dis

tance that the cells fall in a certain time After several hundred sedimentation tests had been charted (when the reading had been recorded every hour for several hours), it was observed that the most constant drop in the curve occurred between the first and second hour Rubin, Morriss, and others observed this fact and considered it the most significant part of the curve Tubes of different diameters when used with the same blood give the same

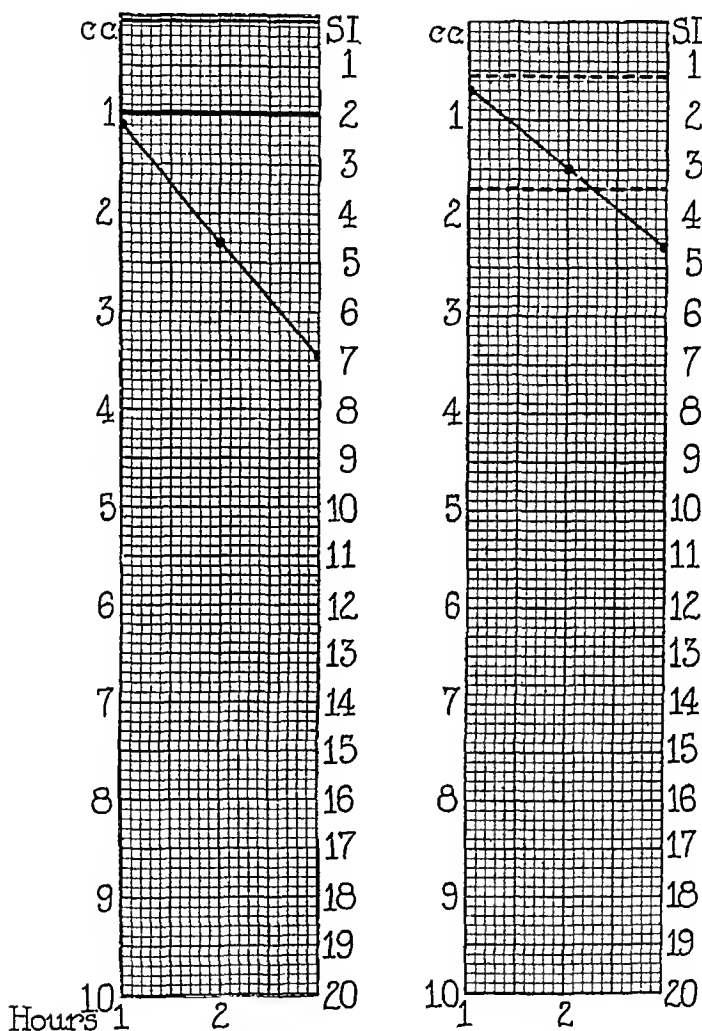


Fig. 2.—Determination of sedimentation index. Values between heavy lines indicate range of normal males. Values between dotted lines indicate range of normal females. SI, sedimentation index.

results With this fact in mind, I devised a method, based on the work of Gilbert, of reporting the sedimentation reaction which would be a combination of time and distance This was called the sedimentation index This seems to me to be a more nearly correct usage of the term index than that of Cutler who in using the term sedimentation index refers to the total sedimentation of the red blood cells at the end of sixty minutes, expressed in

millimeters. According to my method, the sedimentation index can be obtained by the use of either a graph or a table.

The graph paper is standard, authorized by the American Medical Association for its journals and is ruled in one half inch squares each of which is subdivided into twenty five equal squares. However any paper ruled in equal squares would serve as well. The ordinates represent time, each large square indicating a half hour. The abscissa represent volumes of plasma appearing above the falling column of cells, hence the distance the erythrocytes fall. Each large square represents a fall of 0.05 cc.

The sedimentation index (Fig. 2) is obtained by projecting the line drawn between the plotted point of the first and second hours, onto a scale drawn as a perpendicular line two squares to the right of the second hour point or so that the horizontal distance from the first hour point to the second hour point is equal to the distance from the second hour point to the scale. The scale is enumerated with 0 on the horizontal line marked cc, and the integral numbers follow in order downward, each large square representing one unit. The reading is recorded in units and decimals thereof. Thus, a rapid sedimentation rate gives a high sedimentation index.

It will be noted that the method presented here differs from Gilbert's chiefly in that he determines the sedimentation index by drawing a straight line from the 0 point paralleling the greatest drop in the curve during the first hour, to a scale on the first hour line.

SUMMARY

A critical study of methods employed in the sedimentation test has been made. By combining and modifying several tests, a simple and expedient test has been devised. A simple method of reporting results has been proposed, the results being given in terms of a sedimentation index. Average normal values for adult males are from 0.1 to 2.0 and for adult females from 1.1 to 3.4, although it must be stated that in occasional instances there are unexplained variations from the average normal values given. Sedimentation tests by this method can readily be compared with those done and reported by other methods described in the literature.

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DISCUSSION OF PAPERS BY HUBBARD AND GEIGER AND HUNT

Dr Carl Spohr—We have been doing some extensive experimenting in our laboratory on this subject with the view of preparing a paper for this meeting. We have discovered that there are a great many factors which require detailed study. Our method has to do with Sahli tubes. We have used undiluted blood to which an anticoagulant has been added. We place oxalated blood up to the 100 mark in the Sahli tube. It has been our experience that an increased amount of oxalate retards sedimentation. A number of determinations have been made with other anticoagulants. We can state that excessive amounts of anticoagulants will markedly retard sedimentation. Sufficient blood is drawn for several determinations and one portion is allowed to stand from twelve to twenty four hours in the ice chest. It gives a decidedly lower reading. Readings are from 5 to 10 per cent lower than the readings obtained by making the readings immediately after the blood has been withdrawn. If we add citrate salt solution, the cell volume is markedly higher. We have studied a number of cases in gynecology and have obtained some valuable results. Our method was to use a Sahli tube, 2 mg to the cc up to the 100 mark, readings are made in five, ten, fifteen, thirty, forty five, and sixty minutes. These are graphed, the cell volume is also determined.

We have found that the sedimentation velocity in acute active cases is markedly increased, and that we can get satisfactory readings by this method in about thirty minutes.

We have just begun to study this problem and we expect to continue this until we get more definite information. I have given you some of the points which we have really noted that may be of value to you in the future.

Dr Asher Yaguda—About two or three years ago when sedimentation was fast becoming popular in this country I started to do some work with it. I learned that many factors varied the sedimentation time. I took a number of normal individuals who seemed to be in good health and found one of the factors to be exercise. Exercise cut the sedimentation time in half, and a heavy protein diet did the same thing. Some of the other factors were the type of coagulant, and the temperature of the room in which the sedimentation time was taken.

Dr Herman Sharlit—I simply arose to ask, in view of the fact that one of the simple factors would be the specific gravity, if that was specially investigated and if so what the inference was.

Dr H R Brown—I would like to ask a question about the size of the red cells in these experiments. Just what is the practical application in cases of infection? I should like to hear an expression on that subject.

Dr William G Ezton—I would like to ask also if there is any available data on the various elements that must enter into this time drop, such as the specific gravity of the particular cells and particular plasma

Dr Roger S Hubbard—It seems to me that the principal points which should be borne in mind when studies of the sedimentation rate are applied clinically have been very well brought out in the discussion. There are a number of different methods which may be used some of which are better than others but which all measure the same things and are more or less influenced by the same factors. The specific gravity of the blood, the amount of exercise which the patient has taken prior to the test and the degree of anemia present are among what may be called secondary factors which certainly influence the sedimentation rate but are not the ones in which the clinician is interested. A few experiments were carried out to determine whether the effect of anemia could not be overcome. Specimens of blood were centrifugalized and the relationship of the volume of cells to the total volume determined. Artificial anemia was then produced as described in the paper the anemic blood centrifugalized and the cells and plasma mixed in the proportions in which they were present in the original samples. Sedimentation times were then determined on the mixture of cells and plasma. The average of the results obtained agreed well with the sedimentation times determined on the blood as drawn. In a number of individual studies, however, the differences were marked and a method for ruling out the effect of low cellular concentration based upon these experiments therefore cannot be recommended.

Dr A H Sanford—In all of the discussion it seems to me that we have raised the question whether we are not simply dealing with some biologic phenomena that can have after all, very little real diagnostic use. I do not want to offer this as a criticism of Dr Hunt's work, because he is still working on his problem. All phases of it must be studied. At present I think we are being rather hurried into reporting the sedimentation rate of erythrocytes as a diagnostic procedure. The test seems to be principally looked forward to by the gynecologists. That is merely my impression. The gynecologists are insisting that it is a very important test and marked changes do occur in those conditions that have been mentioned. However there is a considerable difference in the sedimentation rate of women's blood as compared with that of men. The physiology of the process needs more study. I am sure.

Dr G B Kramer—It seems to me that the present status of the sedimentation test of erythrocytes is not so much for diagnosis as it is of some aid for the surgeon in deciding when to operate in infectious cases. In our hospital some surgeons believe that in pelvic infections where the sedimentation rate is accelerated operation is contraindicated. It seems to have more value as an aid to prognosis than to diagnosis.

THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS*†

BY FRANCIS B JOHNSON, M D., CHARLESTON, S C

SINCE the introduction of the Kahn precipitation test, many investigations have been made of its usefulness as compared with the Wassermann in the serologic diagnosis of syphilis

We have now ample evidence of its great value as a diagnostic procedure and of its greater saving in time, material, and expense

One of the chief difficulties with the Kahn precipitation test is in the reading of the doubtful and slightly positive reactions. The ability to record correctly the results, with the unaided eyes, depends upon a certain number of factors, of which normal visual perception is of the utmost importance, when there is any defect in vision the poor results are evident. Correction by properly fitted glasses will, of course, obviate this, but even so, considerable experience and practice are required. The holding of the test tube at a certain angle, the degree of light and shadow that strike the tube, is of such importance that each individual has a special light and position that he prefers to make his reading with

It hardly seems necessary to mention the difficulties of the Wassermann, for we are more familiar with them and with the fact that it depends upon a greater number of variable factors and technical details. The Wassermann offers, however, little difficulty in the reading of end-results when made by the method of Kolmer. In regard to the care of technical details, it is essential to be as careful, and to have as thorough an understanding of what you are doing in one serologic test as in another. We should strongly condemn the idea that with the introduction of the Kahn test we have a method so simple and easy, that it can be done by any doctor in his office or by inexperienced individuals without the proper safeguards and controls

The adaptation of the Kahn precipitation test to a method so as to be read by the aid of the microscope has been introduced by Kline and Young.¹ Lately they have added certain refinements in technical details, and it is claimed by them, that it is as specific as the Kahn and Wassermann tests, has the advantage over these methods, in that it is much simpler, requires less apparatus and serum, and is much easier to read than the Kahn test

The technical details, as here given are taken from Kline and Young² for the immediate slide precipitation test for syphilis

TECHNICAL DETAILS

Microscopic slides, two by three inches, are thoroughly cleaned by rubbing with bon ami paste, which is allowed to dry, and rubbed off with a soft cloth. A wire loop, having a diameter of 12.5 mm to 13 mm, wrapped

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with thread and bent at right angles to the stem, is used for making twelve paraffin rings on the slide, by dipping the loop in smoking paraffin, and touching the slide with the loop. All apparatus should be kept warm, about 70° to 80° F, and the air of the room should be fairly moist.

Serums are heated for a half hour at 56° C. With a 1 c.c. pipette graduated in $\frac{1}{100}$, 0.05 c.c., to 0.06 c.c. of each sera is placed in the center of a paraffin ring, allowing the tip of the pipette to touch the glass. Positive control, negative control, and salt control should be used.

Antigen should be prepared, titrated, and mixed for the proper dilution, according to the method of Kahn.² Antigen dilutions should be prepared just before pipetting sera and are usually most satisfactory when used between ten minutes and a half hour after making up. In recent work it has been shown by Kline and Young that a more satisfactory clear antigen is obtained after placing the cholesterolized antigen on ice for one hour then filtering. Through the kindness of Doctor Kline I have been able to check some of his recent antigen with mine prepared in the same way and have found it to be preferred to those that were filtered without chilling. One drop of antigen dilution is added to each sera by the use of a capillary pipette, dropping 0.0075 to 0.0085 c.c. to the drop. In earlier tests 0.015 c.c. was added. Each sera and antigen is mixed by the aid of the broad end of a wooden toothpick. After the completion of adding the antigen to the twelve rings, the slide is taken between the thumbs and index fingers of both hands and by a rocking circulatory movement further mixing is done for two to three minutes. Any spilling from the chamber makes the reaction unsatisfactory and that particular serum should be retested. Tests are better done in duplicate, by using different antigens so as to have a check on the results.

All readings should be made by the use of the microscope with low power objective and 10 X eyepiece with a dim light. Results are recorded in pluses, according to degree of clumping and size of clumps. A clear fluid is negative. Fine granular clumping is +, a fine flocculation is ++, a marked flocculation is +++, and a coarse flocculation is ++++.

COMPARATIVE RESULTS

In a series of 1500 tests, I have made a comparative study of my results of the microscopic slide precipitation test, by the method of Kline and Young,¹ with the three tube Kahn test,³ and with the Wassermann test.

The Wassermann tests were made by the overnight fixation method of Kolmer⁴ using 0.1 c.c. and 0.05 c.c. of serum and 0.1 c.c. of serum control. With the Wassermann, inactivation was for fifteen minutes and natural antisheep hemolysins were not removed. For the tube Kahn and the microscopic slide test, the sera were heated for one half hour. The same antigens were used for the Kahn and slide tests and the dilutions were titrated and prepared according to the method of Kahn, but were made up separately. Readings were carefully checked by two, and sometimes three, experienced individuals.

In Table I are seen the results of fifteen hundred comparative microscopic Kahn tests, Kahn tube tests, and Wassermann (Kolmer) tests.

TABLE I

FIFTEEN HUNDRED COMPARATIVE MICROSCOPIC KAHN, TUBE KAHN, AND WASSERMANN
(KOLMER) TESTS SHOWING AGREEMENTS

	TESTS	PER CENT
Agreement of all three	1335	89 0
Agreement of microscopic tests and Kahn	1445	96 3
Agreement of microscopic tests and Wassermann	1370	91 3
Agreement of Kahn and Wassermann	1442	96 1
Microscopic tests positive	536	35 7
Kahn positive	513	34 2
Wassermann positive	459	30 6

It will be noted that a uniform agreement of all three was found in 89 per cent. I can find no other comparison of the three methods at the same time, but it is evident from this that there is a fairly close agreement between the three. The agreement of the 96 3 per cent of the microscopic tests with the tube Kahn compares favorably with the findings of Kline and Young, in their preliminary report of 268 comparative microscopic tests with the Kahn tests in which they found an agreement of 97 7 per cent. The agreement of the microscopic tests with the Wassermann did not compare so favorably with that of Kline and Young, who in their first series found an agreement of 92 3 per cent, and in their later series, with improved antigen, an agreement of 97 6 per cent. This will be considered more fully in discussing the disagreements.

It is of interest to mention that my agreements of the tube Kahn with the Wassermann, in this series, was 96 1 per cent, whereas in a previously reported comparison of six thousand tests,⁵ the agreement was 90 7 per cent. From the comparison of the positives by the three methods, it will be noted that the greatest percentage of positives is obtained by the microscopic test, and the least number of positives by the Wassermann.

In Table II are shown the disagreements of the three tests, including anticomplementary Wassermann tests, together with a comparison of those disagreements in which syphilis could be clinically diagnosed.

TABLE II

FIFTEEN HUNDRED COMPARATIVE MICROSCOPIC KAHN, TUBE KAHN, AND WASSERMANN
(KOLMER) TESTS SHOWING DISAGREEMENTS

	TESTS	CLINICALLY SYPHILIS	PER CENT CLINICALLY SYPHILIS
Total Disagreements	165	127	76 9
Micro Pos, Kahn Pos, Wass Neg	64	47	28 4
Micro Pos, Kahn Neg, Wass Pos	8	8	4 8
Micro Pos, Kahn Neg, Wass Neg	37	25	15 1
Micro Neg, Kahn Pos, Wass Pos	6	6	3 6
Micro Neg, Kahn Neg, Wass Pos	8	8	4 8
Micro Neg, Kahn Pos, Wass Neg	10	6	3 6
Micro Pos, Kahn Pos, Wass A C	24	23	13 9
Micro Neg, Kahn Pos, Wass A C	1	1	0 6
Micro Pos, Kahn Neg, Wass A C	4	2	1 2
Micro Neg, Kahn Neg, Wass A C	3	1	0 6
Microscopic Test Positive	137	105	63 6
Tube Kahn Positive	105	83	50 3
Wassermann (Kolmer) Positive	22	22	13 3

In the class of patients coming to our hospital and out patient clinic the greater majority of which are negroes, it is very difficult to obtain a reliable history, so that those placed as clinically syphilis are from definite evidence or previous hospital history showing definite evidence.

It may be of interest to state that, in more than thirty thousand Wassermann tests made in Charleston, S C about 45 per cent positives are found among the negroes to 15 per cent positives among the whites. This of course, represents repeated tests on cases under treatment as well as single tests.

It must be understood that the thirty eight cases of disagreements excluded do not mean falsely positive tests, but that no definite history of syphilis was obtainable. Among the disagreements, the microscopic test was positive in 63.6 per cent. The Kahn was positive in 50.3 per cent, and only 13.3 per cent gave a positive Wassermann test, this latter was due to the fact that thirty two of the disagreements were due to anticomplementary tests, twenty seven of which have given an undoubted history of syphilis.

The results with the microscopic test in treated cases of syphilis have shown the same finding that the tube Kahn has i.e., a greater number of positives are obtained by these methods than with the Wassermann test, showing the increased sensitiveness of the test.

From as careful a study as possible to obtain a reliable history, I could find no evidence that positive tests were obtained in cases definitely proved not to be syphilis. That is, none could be proved falsely positive.

I have used the microscopic precipitation test with cerebrospinal fluids, using the method of globulin concentration⁶ and have so far obtained practically parallel results with the tube Kahn and Wassermann tests. The number is still too small to make any decided statement in regard to its value with cerebrospinal fluids. However for your information results are here shown in Table III.

TABLE III
CEREBROSPINAL FLUIDS COMPARATIVE MICROSCOPIC KAHN TUBE KAHN AND
WASSERMANN TESTS

			NUMBER TESTS	
Agreement of all three			23	92%
Positive Agreements			3	All syphilis
Disagreements			2	8%
Micro Pos, Kahn Neg	Wass Pos		1	Syphilis
Micro Neg, Kahn Neg	Wass Pos		1	Syphilis

My thanks are here expressed to Dr W G Gamble and Dr S Simons Miss Eleonor Townsend, and Miss Lois Thompson for their valued assistance in this investigation.

CONCLUSIONS

The number of comparative results reported so far with the microscopic precipitation test are too few upon which to form any decided opinion. My results, however, compare favorably with those given by Kline and Young, and I believe confirm their findings as follows:

1 The microscopic slide precipitation test is simpler to perform than the Kahn tube test, requires less material and time, and the results are more easily read

2 The microscopic test is slightly more sensitive than either the Kahn or Wassermann tests and appears to be as specific

3 The microscopic test appears to be as adaptable as the Kahn test to the globulin concentrated cerebrospinal fluids and checks favorably when compared with the Wassermann test

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A COMPARISON OF THE KOLMER AND KAHN TESTS FOR SYPHILIS*

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IN THE past two or three years numerous articles have been written comparing the Kahn test with the so-called Wassermann test, this latter term being used to cover all systems employed or all pet methods used. We make this statement in view of the fact that until an attempt at some sort of standardization was made about three years ago, there were probably as many methods employed as there were serologists doing the test, and all with more or less success. However, when comparison is made between the Kahn and the complement-fixation test for syphilis, the method employed should always be given. It has been the experience of many laboratory workers throughout the country, as evidenced by statements made in regard to the Kolmer complement-fixation test at the annual meetings of the clinical pathologists, that the Kolmer test is perhaps superior to many of the methods used heretofore. So true is this that many of us have abandoned our pet methods in favor of the more delicate and reliable Kolmer method. In a number of the papers that used the term *Wassermann test*, the antigen has been mentioned, but in many more perhaps, only the term Wassermann test has been employed.

During the past eight months we have been interested in a comparison of the Kolmer complement-fixation test and the Kahn flocculation test in treated neurosyphilis at the Veterans' Bureau Hospital, No 100, Camp Custer, Michigan. While the number of cases studied is small, we feel that our results are worth reporting at this time. Due to the relative frequency with which articles have appeared in the literature concerning the superior value of one or

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From Veterans Bureau Hospital No 100

the other of these tests, we decided to investigate each, relative to its merit as a guide to the treatment for syphilis. The number of cases selected is small, and upon many we did not have an entrance test with both methods due to the fact that the Veterans' Bureau Hospital No. 100 was established about two years ago, but the laboratory was not organized until about a year later. We shall show the results obtained by both the Kolmer and the Kahn method after one, two, three, four, five and six courses of treatment. We are also running parallel with the Kahn and Kolmer tests the technique adopted by the Veterans' Bureau. The results of this comparison will be published at a later date.

The objection voiced, to the Kolmer as well as to many other ways of doing complement fixation tests for syphilis is that of the consumption of time, referring particularly to overnight incubation in the ice box. This, however to our minds is a more or less groundless objection because time is not the most important factor in the serologic diagnosis of syphilis. In most instances we find syphilis has existed many days, months or years before patients come to a doctor for advice. Therefore what great harm can be done in further delaying the report on the blood serum for twenty-four hours when we know at the time that we are perhaps going to have the most reliable result that can be obtained. In other words, accuracy should never be sacrificed for speed. In many instances the same physician who expects us to hurry through a twenty-four hour test in an hour or so for a disease like syphilis will sit by, in an acute infection like diphtheria and wait for a report on the culture until the next morning.

Now the question constantly arises as to the advisability of substituting the Kahn test for the complement fixation test for the diagnosis of syphilis and the control of the treatment. A very brief review of the opinions of several workers of the country who have been comparing the Kahn test with other methods might serve as an introduction to our experiences.

Owen found 93.8 per cent of 1,600 cases to agree and he also found his divisions almost wholly in treated cases and considers the Kahn a valuable check in a small percentage of cases.

Giordano finds it valuable in emergency tests for donors for blood transfusions, but at the same time he explains the difficulty in reading a weakly positive reaction. He does not like to report a weak reaction unless it is checked by the complement fixation test. In view of this statement it would seem to us that if it cannot be relied upon when it is not checked by the complement fixation test it certainly should not be relied upon alone at all.

Kelly found them to agree in 94.45 per cent of 110 cases. He calls attention to increased flocculation when left in the ice box overnight. We have had this experience and have found that false positives may be obtained in this way.

Greenbaum found the Kahn to run positive in many cases after the Kolmer became negative and vice versa, and he advises that both methods be used. In our experience the Kahn becomes negative much sooner than the Kolmer.

Haven and Taylor conclude that the present value of this test is questionable as a Public Health laboratory method

Lederer, in comparing it with the Veterans' Bureau Wassermann method, finds the Kahn test to be of value, and combining both tests simultaneously is the ideal diagnostic practice

Becker concludes that the Kahn gives more uniform results in repeated examinations and is more difficult to change from positive to negative by treatment

Anderson concludes that "the great reliability of the Kolmer method and the simplicity and apparent reliability of the Kahn precipitation test made this combination the best standard procedure for the diagnosis of syphilis "

Kilduffe finds that the Kahn test checks with the Wassermann reaction in from 80 to 90 per cent of cases, depending upon the care with which it is performed and the delicacy and reliability of the Wassermann technic with which it is compared and that false negatives and false positives occur with the Kahn test

Houghton in an article in the *Journal of the American Medical Association*, December 4, 1926, declares the Kahn test has removed the serum diagnosis of syphilis from empiricism and has placed it upon the basis of quantitative science, and in his summary calls attention to the fact that the Kahn test is more sensitive than the Wassermann test in treated cases. We feel that the former statement remains to be proved, and our experience with the following few cases of treated syphilis tells us that the second statement does not hold

From the foregoing it seems that we have a few who advocate the Kahn test alone, more who advocate the Kolmer test alone, and a much larger number who emphatically advise the use of both tests as being the nearest to perfection

Our figures in the short series we have ready at this time are as follows

After one course of treatment

8 cases		
Kolmer positive in 4	50 %	} Agreement 50%
Kahn positive in 0	0 %	
Both positive in 3	37 5%	
Both negative in 1	12 5%	

After two courses of treatment

15 cases		
Kolmer positive in 6	40 %	} Agreement 46 6%
Kahn positive in 2	13 4%	
Both positive in 4	26 6%	
Both negative in 3	20 %	

After three courses of treatment

11 cases		
Kolmer positive in 4	36 3%	} Agreement 54 5%
Kahn positive in 1	0 9%	
Both positive in 4	36 4%	
Both negative in 2	18 2%	

After four courses of treatment

10 cases		
Kolmer positive in 6	60 %	} Agreement in 40%
Kahn positive in 0	0 %	
Both positive in 2	20 %	
Both negative in 2	20 %	

After five courses of treatment

18 cases		
Kolmer positive in 7	38 8%	} Agreement in 55 4%
Kahn positive in 1	0 6%	
Both positive in 7	38 8%	
Both negative in 3	16 6%	

After six courses of treatment

14 cases		
Kolmer positive in 5	35 7%	} Agreement in 56 7%
Kahn positive in 1	7 0%	
Both positive in 5	35 7%	
Both negative in 3	21 0%	

After seven courses of treatment

13 cases		
Kolmer positive in 5	38 5%	} Agreement in 61 5%
Kahn positive in 0	0 %	
Both positive in 5	38 5%	
Both negative in 3	23 %	
Kolmer positive and Kahn negative in 37	or 41 5%	} Agreement in 52 8%
Kahn positive and Kolmer negative in 0	or 5 6%	
Both positive in 30	or 33 7%	
Both negative in 17	or 19 1%	

In conclusion we wish to say that our position in this matter is neutral. We do not believe we are in a position to substitute the Kahn test for the Kolmer complement fixation test at this time. In our minds the Kolmer test is more reliable and sensitive for the diagnosis of syphilis and as a prognostic indicator in treated cases. We believe, however, that the Kahn test is worthy of merit, and both tests should be used simultaneously until one or the other is eventually discredited.

DISCUSSION OF PAPERS BY KILDUFFE,* JOHNSON AND RODERICK
SALISBURY AND CATES

Dr R. L. Kohn—It is difficult in a short space of time to talk extensively on precipitation in syphilis. The problem is too big. I was interested in the conclusions of the speakers that while the Kohn test is reliable it should be used with the Wassermann test. I am not recommending the abandonment of the Wassermann test; neither am I inviting anyone to use the Kahn test. Indeed, there is not a man or woman in this room or anywhere, whom I have ever asked to study this test. Those who have come to us at Lansing or have written me about the test, I have assisted gladly, but beyond that whether one uses the Wassermann or the Kahn or both or any additional tests the problem is outside my hands.

I recognize that it is not a simple matter for many of you to turn from complement fixation tests to which you have devoted perhaps ten or fifteen years and turn to the problem

of precipitation On entering this room, I met a man who, about twelve years ago, began to point out the importance of titrating complement A good many of you, like Dr Ottenberg, gave much time and effort in developing improvements in the Wassermann test It is not to be expected, therefore that you would hastily abandon this work, but when the time comes and you decide to give the Kahn test a trial, I would urge you to do it correctly and not crowd it to the wall

My experience has been, almost invariably, when the Wassermann and Kahn tests are used together that the latter does not get a fair chance After giving much time and energy to the Wassermann test, laboratory workers find the additional test burdensome and do not carry it out correctly Many of our visitors in the Michigan Department of Health come with a record of several years' experience with the Kahn test Yet, when they see our work, they invariably admit that in many details they have not followed the correct technic The thought that I wish to leave with you is not to please try the test, but when you finally decide to try it, to do it correctly Obtain proper glassware and apparatus See that your worker picks up the correct method in some dependable laboratory Obtain some standardized antigen from a reliable biologic house or from the Michigan Health Department at Lansing If you prepare your own antigen, let us help you in its standardization Then it will take very little time, and you will be convinced of the practicability of the test, of its reliability as a diagnostic agent in syphilis and of its general superior qualities

A word regarding antigen Its standardization should be in the hands of expert serologists in central laboratories I should like to suggest respectfully that possibly your organization might look into this antigen problem with a view of supplying the standardized product to your members who are removed from medical centers If you could arrange with some biologic house, for example, to provide standard Kahn antigen to your members at some minimum cost, it would go a long way toward good results with the Kahn test by your group, and I would be glad to assist you in any way possible I hardly need add that should your organization care to appoint a committee to study this test or should you designate certain laboratories to study it, I shall be glad to be of any assistance, should I be called upon for suggestions

I should also like to bring to your attention the fact that the Kahn test embraces a series of procedures and in this regard opens up a new field for the clinical pathologist Aside from the routine or diagnostic procedure, there is the quantitative procedure, which is of special value in specific therapy, the presumptive procedure, which is more sensitive than the routine test and is of value in special cases where this test is negative, the procedure with syphilitic fluid other than serum and the microprocedures, also the qualitative and quantitative procedures for spinal fluids I venture to predict that several years hence, the public health laboratory will limit itself to the routine Kahn test, and the various additional procedures will be carried out and interpreted by the clinical pathologist

Dr Kline—I would like to take up the discussion exactly where Dr Kahn ended There is great promise in the precipitation test for syphilis Dr Kahn has not only focused the attention of serologists of this country on this method of detecting syphilis, but he has also worked out additional principles for the test, especially demonstrating the importance of using concentrated antigen

Our experience in this field began two and a half years ago when Dr Kahn kindly assisted us in many ways to acquire the technic I would like to express again my appreciation for his help After doing a number of Kahn tube tests, we decided to try doing the tests by the open slide method We had been using the open slide method of blood typing reported by Vincent during the war and had found it a satisfactory method for Widal's test and other bacterial agglutinations also We did these tests on a microscopic slide with paraffin rings mounted on it to confine the ingredients After determining proper sized rings and quantities of antigen and serum to be used, we found that the precipitation test for syphilis could also be done with accuracy by this simple method At first we used the antigen advocated by Kahn, but finding it too sensitive to cold and to time, we tried other methods of preparation, and for the past several months we have been using an antigen that works as well at low temperatures as at ordinary room temperature and is a stable lipoidal

suspension in contrast to the unstable one of Kahn. One such indicator for syphilis, prepared March 30, still possesses its antigenic properties.

Concerning the mechanism of the precipitation test for syphilis Weil's hypothesis that antibodies in syphilis are formed against body cell substances liberated by the destructive activity of *Spirochaeta pallidum* rather than by the spirochetes themselves will apply as well as it does to the Wassermann reaction. In fact Wassermann in 1921 claimed that the substance resulting from the union of the lipoidal antigen (lecithin) and its antibody should be called the Wassermann aggregate and that this aggregate is capable of doing two things: (1) binding complement and (2) causing precipitation as in the Sachs and Georgi test. In the precipitation test for syphilis it seems that a hydrophilic colloid (lecithin) protects cholesterol (hydrophobic colloid) against precipitation by water unless some substance is present (in the patient's serum) that combines with the lecithin and interferes with its protective action when the cholesterol will precipitate out and give a positive reaction.

It is important to test out this theory for if it is true and the simple indicator cholesterol can be used instead of the hemolytic system, then there is a possibility that a simple standard test for syphilis can be devised.

Dr H. A. Hesse—Is one of these poor, tired serologists. I would like to add something that is a little time saving device in the use of the quantitative Wassermann. The Kahn test is completed, and then quantitative Wassermann tests are performed only on those serums which give a positive Kahn reaction; the other serums being run by the ordinary qualitative technique.

Dr Robert I. Kilduffe—The particular point in my paper upon which I wished to focus attention was the present propaganda for the exclusive use of the Kahn test. Dr Kahn has told us that he has never by word or published utterance advocated its exclusive use. I feel, however, that the fact that it has been adopted as an exclusive test by the laboratories of which he is the serologist is an expression of opinion on this point and I cannot recall that Dr Kahn has ever expressed any disapproval of its exclusive use or that he has ever suggested caution in so applying his test.

Dr Kahn tells us that he has been written to by laboratories having the exclusive use of his test in mind. It would be interesting to know what he has replied if he has said that he considered this hasty or unwise or if he simply acquiesced, expressed satisfaction and offered a supply of antigen?

I consider it unfortunate also that this test has been consistently exploited and demonstrated almost exclusively before county medical societies to whose members the idea that all that is necessary is a pipette, a test tube and a bottle of antigen is naturally attractive. The fact that as I am told by a colleague at a recent meeting of the American College of Physicians two physicians expressed the intention of spending one hour in a laboratory to learn the Kahn test so as to use it in their offices is evidence that the impression is abroad that anyone may learn this test in a short time.

This is one of the impressions I desire to combat and therefore I want to emphasize again that I do not by any means agree with those who disseminate it.

I think if this Society, representing as it does a cross section of laboratory workers, were to express its opinion as to the Kahn test, whether it should be considered an additional valuable method, or sufficient as an exclusive method in the serological diagnosis of syphilis, the opinion would carry great weight and would be an excellent thing for all concerned, not least among whom is the patient!

Dr Robert A. Acuity—I had the pleasure of starting with Kolmer in his original work. I believe I have heard Dr Kahn's original presentation of his paper.

The future work of the Veterans' Bureau, which is now only beginning, will play an important part in this.

In fairness to the patient the diagnosis of syphilis is going to be very important for instance a diagnosis of syphilis alone a clinical diagnosis of paresis being rather difficult.

I think we have spinal fluid absolutely conquered. In the case of the blood Wassermann we should have just as much evidence as we can have in that data. I agree with Dr Kilduffe that all of them should be used.

Dr Francis B Johnson—When we consider that the Wassermann test was introduced some twenty years ago, and realize that it took time and various modifications before its acceptance at its true value, we should give more consideration to the Kahn test and its modifications. These tests have comparatively only recently been introduced. While certain clinical pathologists and others realize, to their satisfaction, that it is of equal or greater value than the Wassermann, there are still many others that are not convinced, and until the majority are convinced, we have no right to give up the use of the Wassermann to some other test. The Wassermann repeatedly proves itself of value in cases with a negative Kahn and vice versa, therefore, we should do them all, for the best aid to diagnosis. I believe the Kahn is more sensitive than the Wassermann. If the one plus Kahn is compared to the one plus Wassermann, the Kahn is not as specific.

Dr Kahn (closing)—One of the speakers brought up an interesting point. How is it, if I do not advocate the abandonment of the Wassermann test, that I, as "director of laboratories" of the Michigan Department of Health, rushed and abandoned the Wassermann test and made the Kahn test standard? In the first place, I do not "advocate" anything. I am not an advocate by nature or by training. My problem of late years has been to learn something about the precipitation phenomenon in syphilis, and with the accumulation of some facts, we gradually evolved what seems to be a practical and reliable method for the serum diagnosis of syphilis. This method was reported to Michigan physicians from July, 1922 to October, 1925, parallel with the Wassermann test. A total of 175,000 Kahn tests were thus reported. Dr C C Young, the director of laboratories with the other administrative officers of the Michigan Department of Health, felt that the time had come to abandon the Wassermann, because the Kahn has consistently proved itself both more specific and more sensitive than the Wassermann test. It was a purely administrative problem and outside of my hands.

My interest in connection with outside workers is to help them do the Kahn test correctly. Beyond that my interest ends. Whether or not any one will continue to perform the Wassermann test after he has become expert with the Kahn test is an individual problem.

This I should like to leave with you, namely, that there is little question but that we are dealing in syphilis with one substance which can be detected either by precipitation or by complement fixation. Recently we have completed a series of studies, wherein we added the hemolytic system to completed Kahn tests. In other words after reading the precipitation results we added complement antibody and cells and read the complement fixation results. In only 2 per cent of cases did we find disagreement between the two readings. What is well to remember is that the hemolytic system is merely an indicator and is in no way related to syphilis.

FATALITIES FOLLOWING THE USE OF ARSPHENAMINE*†

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INTRODUCTION

SEVERE and undesired reactions following the administration of arsphenamine fortunately are not common. Their infrequency, however, should not cause one to overlook the fact that very dangerous and even fatal results may occur at the most unexpected moment.

Various forms of inorganic arsenic have for years been used in the treatment of disease. The concentration of these drugs however necessary to destroy the disease producing parasites was in all cases harmful to the host. Therefore, the attempt of experimentation was to secure a compound of arsenic which would kill the parasite and yet be in such form or concentration that it would be relatively innocuous to the host. The first of these compounds was atoxyl or sodium arsinate. This compound served its purpose but was still too toxic for general use. Ehrlich in his chemotherapeutic studies discovered para dihydroxy meta diamino arsenobenzene or salvarsan. Later, by the addition of formaldehyde to the formula he developed neosalvarsan. More recently Jacobs and Heidelberger¹ have prepared a derivative of atoxyl, triparsamide. This substance was very thoroughly tested by Brown and Pearce² and is at the present time recognized as a valuable drug, especially in the treatment of neurosyphilis. Within the last few years Loevenhart and his coworkers have prepared many new organic arsenicals and have tested their efficiency in syphilis and related conditions. The very general use of organic arsenicals proves that they have fulfilled the demands of the situation, namely, that of (a) being an efficient parasiticide (b) being relatively harmless to the patient, and (c) being easy and safe of administration. There are, however, a certain few who possess an idiosyncrasy and in whom more or less severe reactions occur. Such reactions vary widely in their form and intensity but are uniformly unexpected and undesirable.

CASE HISTORY

C A, aged sixteen white female, was admitted to the venereal clinic four weeks previous to death. She had been given three injections of nearsphenamine. On May 29 1926, an injection of 0.6 gm of nearsphenamine was given with no immediate untoward effects. Six other patients were given injections of the same lot of drug and experienced no ill effects. On May 31 or two days after the injection the patient complained of a slight headache and malaise. This malaise and headache became very severe in the evening, and early on the morning of the next day she became comatose. There was no nausea or vomiting at any time. On June 1, or three days after the injections another one of the patients became ill with the same symptoms. This latter patient became comatose the same after

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noon Both patients presented the same clinical symptoms, namely, headache, malaise, and coma, both received neocorphenamine intravenously and were taking iodides by mouth, but no mercury had been used in either case. In the terminal twenty-four hours of life in both cases there was a complete anuria. The first patient died on June 2 and the second on June 1.

Through the kindness of Dr. C. P. Robbins, of Columbus, Ohio, we were able to perform an autopsy upon the first patient with the following findings. Upon opening the body, an inspection of the abdominal cavity showed that the subcutaneous fat was normal in amount. The omentum was large, and contained a considerable quantity of fat. The liver was slightly below the costal margin but not noticeably enlarged. The surface of the right lobe was darker in color than the remainder of the liver and had a somewhat dry, wrinkled appearance. The appendix was retrocecal, about 1 inches long, and slightly larger in diameter than normal, it showed no evidence of recent inflammation. Within the pelvis the peritoneum surrounding the uterus was covered by a bloody fluid, this fluid was easily removed and the peritoneum itself was of normal color. The urinary bladder was contracted and empty. In the left side of the peritoneal cavity in the region of the spleen, there was a large amount (4 or 5 ounces) of bloody fluid, similar to that found in the pelvis. The right side of the peritoneal cavity was entirely dry and normal in its appearance. There was no evidence of peritonitis or inflammatory exudate in any portion of the peritoneal cavity.

Thoracic Cavity—The anterior margin of the left lung was prominent and apparently emphysematous. In the left pleural cavity there was a large quantity of bloody fluid similar to that seen in the peritoneal cavity, this fluid was somewhat thicker than the usual pleural exudate and was of a somewhat slimy mucoid character. On removal there was found to be approximately one quart of this fluid. The right pleural cavity was perfectly normal in its appearance. The thymus gland was distinguishable. The anterior mediastinal glands were not visible. The pericardial sac was normal in appearance and contained the usual amount of clear fluid. The left lung was of normal size. The pleura, especially of the posterior portion, was a deep red color but did not show evidence of inflammatory change or exudate. The lung was crepitant throughout, although the lower lobe was slightly firmer in texture. There were no areas of consolidation. On section the lower lobe was edematous and was of a deep red color. Crepitation in this portion was somewhat limited. The vessels at the root of the lung were free of clot. The bronchi were normal in appearance with a slight congestion of the mucous membrane. The right lung was crepitant throughout, although the posterior portions were slightly firmer and of a darker color. The vessels were free and unobstructed. The bronchi contained a small amount of frothy mucus, and the mucous membrane was congested. Section through this lung showed an edematous condition of the posterior portions of the lower lobe. The heart was of normal size and appearance. On opening the heart the cavities seemed to be of usual size and appearance, and the valves were all normal in appearance. The myocardium was of the usual color and consistency. The aorta showed a very slight atheromatous change at its base and also in the transverse arch and in the abdominal portion.

Organs of the Abdominal Cavity—In attempting to remove the spleen, the bloody fluid surrounding it was removed and an inspection of this region revealed the fact that a large portion of the cardiac end of the stomach was absent and that the diaphragm immediately to the left of the vertebral column was also missing. Examination of these organs showed that the stomach wall and diaphragm had both disappeared because of necrosis and sloughing and that the fluid which surrounded the spleen and filled the left pleural cavity consisted of stomach contents mixed with blood. The spleen itself was of normal size, its capsule was of a dark reddish color. On section the spleen was darker at its lower lobe. There was no evidence of necrosis, however, either about the capsule or in the spleen substance. The tail of the pancreas was darker in color and somewhat softer than normal, this portion being surrounded by the fluid in the vicinity of the spleen. Other portions of the pancreas were normal in their appearance and consistency. The adrenals were normal in size and appearance. The kidneys were similar in appearance, their capsules stripped very easily. The surface of the cortex was normal in color and smooth. On section the markings were distinct and the pelvis were normal. The lower pole of the

left kidney was somewhat darker in color than the remaining portion. The ureters were both normal in appearance. On inspection it was found that a large portion of the posterior surface of the cardiac end of the stomach had sloughed away; the margins of the slough were very necrotic and thin. The stomach contents had escaped through this sloughed portion into the peritoneal cavity and a slough of the left side of the diaphragm had allowed the stomach contents to pass into the left pleural cavity. By measurement there was approximately 6 inches of the stomach wall missing, and the opening into the diaphragm measured 3.5 inches. Upon opening the stomach there were no signs of ulceration or of local inflammatory change. The mucosa of the pyloric portion was somewhat congested but otherwise of normal appearance. The posterior surface of the stomach wall was covered with tenacious mucus. The intestines were distended and were normal in their appearance. Examination of the mucous membrane of the entire small intestine and colon failed to reveal any areas of marked congestion or hemorrhage. The uterus was of normal size and consistency. The right fallopian tube was somewhat enlarged and was thin walled. On section there was seen a bloody serous fluid; there was no evidence of pus. There was a small single cyst on the right ovary measuring one half inch in diameter. The left fallopian tube was also somewhat larger than normal; its walls were thin; the fimbriae were shortened. The ovary measured 2 inches in diameter and contained several small follicular cysts. On opening the uterus it was seen to contain a small amount of blood tinged mucus in the fundus. Otherwise it was normal in appearance. The liver was of usual size and consistency. On cut section the tissues appeared somewhat darker than normal but the liver contained only a small amount of blood. The gall bladder was normal in size and moderately filled with bile, but contained no calculi.

Examination of the bony portions of the trunk including the vertebral column, the ribs and the bones of the pelvis showed no evidence of pathologic change.

The Head.—The scalp calvarium and the dura mater were all normal in appearance. The pia mater over the surfaces of the hemispheres was much congested; the veins and capillaries being very distinct. The brain was of normal size. The convolutions were full and the sulci somewhat inconspicuous. There was no evidence of meningitis or of inflammatory exudate. After removal of the brain, examination of the vessels at its base showed no evidence of vascular disease. On the inferior surface of the right temporal lobe there were a few very indefinite granules resembling miliary tubercles. On section into the hemispheres of the brain, small, dark, apparently necrotic areas were encountered in the roof of each lateral ventricle. Examination of the bones at the base of the skull showed no evidence of pathologic changes. Examination of the brain made in the laboratory revealed that there were other softened necrotic areas involving the pons, the crura cerebri and the posterior portion of the optic thalamus; these areas resembled those seen at the time of the postmortem involving the tissues of the wall of the lateral ventricles. The most extensive hemorrhage was seen in the pons.

The anatomic diagnosis was that of (1) congestion, capillary hemorrhage, hyaline thrombosis and edema of the brain, (2) postmortem autolysis of the stomach wall and diaphragm, (3) edema of the posterior portions of the lungs, and (4) acute tubular nephritis.

The microscopic examination of sections taken from the degenerative region in the brain showed that the pathologic changes consisted of very intense congestion with small hemorrhages and edema. These changes were most pronounced in the pons but were very definite in the other involved portions of the brain. The walls of very many of the congested vessels showed an infiltration with lymphocytes and polymuclear leucocytes. This infiltration apparently involved the vessel wall and was not within the perivascular lymph spaces as seen in the vascular changes of encephalitis lethargica and in poliomyelitis. Sections from the kidney showed a very pronounced tubular nephritis involving the epithelium of all the convoluted tubules and Henle's loop. Sections of the stomach taken near the margin of the slough showed a gradually increasing degeneration, and autolysis of the cells at the edge of the necrotic area was approached. Sections from the remaining organs showed no definite pathologic change.

Chemical examination of the liver revealed the presence of small quantities of arsenic. Toxicity tests on ampules of neosalvarsan from the same lot used, showed that they conform to the recognized standards.

CLASSIFICATION OF REACTIONS

The British Medical Research Council³ in their report sum up the reactions which may follow the administration of organic arsenicals as follows:

- "1 Immediate reactions consisting of diarrhea, vomiting, pyrexia, and headache. Under this group are also classed the so called vasomotor phenomena and the anaphylactoid and nitritoid crises.
- "2 Effects on the nervous system. The most important of these is encephalitis hemorrhagica.
- "3 Effects on the liver. These are clinically grouped into:
 - "a Early jaundice—coming on in a few days, usually mild and evanescent but rarely more severe and persistent.
 - "b Late jaundice—not earlier than several weeks of a more severe and prolonged type.
 - "c Acute yellow atrophy of the liver, supervening on late jaundice.
- "4 Exfoliative dermatitis and slighter skin reactions. The former may be complicated with bronchopneumonia or septicemia and end fatally.
- "5 Various rare lesions, such as acute hemorrhagic nephritis, ulcerative enteritis, and aplastic anemia.

"In addition to these complications, there are a number of other conditions which were previously believed to be due to relapses of the disease or the sudden liberation of toxins from the killed treponema. In this group are the so-called 'Heidenheimer reaction,' deafness, cranial nerve palsies and other forms of 'neuropsychence'."

INCIDENCE

Statistics upon the explainable fatalities following the use of organic arsenicals are unsatisfactory. Phelps⁴ cites Lake, of the United States Public Health Service, as stating that he had collected thirteen deaths following the use of arsphenamine and the same number following the use of neoarsphenamine in the United States. In the United States Navy, from 1919 to 1923, out of 15,170 cases of syphilis treated with an unknown number of injections, there were fourteen deaths. Phelps cites German statistics as stating that there was one death for every 3,788 cases treated with 10,984 injections. The British Medical Research Council report two sets of statistics, Hospital A, one death in 1,629 cases with 13,000 injections and Hospital B, one death in 375 cases treated.

From these statistics, it becomes apparent that there is one unexplainable fatality in each 5,000 to 10,000 injections. Phelps, however, states that with neosalvarsan there should not be more than one fatality in every 40,000 injections.

THEORIES OF ETIOLOGY

From the papers of Ehrlich, Bernard, Danysz, Warthin and Schamberg, the theories of the etiology of these fatalities may be summarized as follows:

1 Due to previous or concurrent kidney disease, there is a faulty elimination of the drug, with a resultant arsenic poisoning

2 That it is essentially a Herxheimer reaction due to the sudden liberation of a large amount of endotoxin from the killed treponema

3 That it is due to the destructive and dilating action of arsphenamine on the blood vessels which is not counteracted by adrenalin, which is deficient perhaps because of arsenical destruction of the adrenals

4 That the injection of arsphenamine into the blood stream causes a precipitation of the blood proteins and consequent multiple emboli

Pearce and Brown report that in their experiences following the use of salvarsan, hemorrhage and congestion of the kidney congestion, hemorrhage and necrosis with degeneration and a reduction of the chromaffin content of the adrenals are found Kolmer and Luck⁶ found that the injection

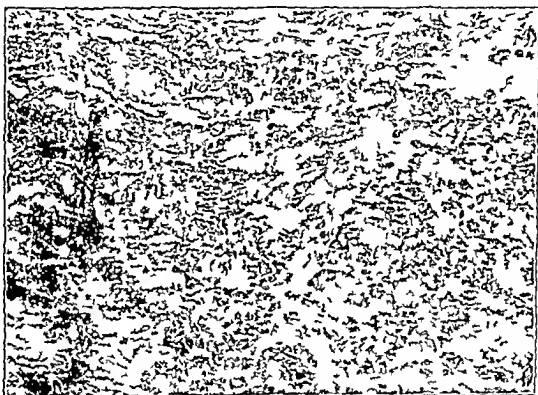


FIG 1—Low power of area of multiple capillary hemorrhage in the pons

of acid solutions of arsphenamine produced widespread vascular injury. Single overdoses of the disodium salt produced severe vascular and tissue alterations, particularly in the liver, kidney, suprarenals, and spleen. Repeated therapeutic doses produced no effect. The same results, except of a milder form, were secured with neosalvarsan. Jackson and Smith⁷ from their pharmacologic experiments with salvarsan and neosalvarsan, came to the following conclusions: (1) slow injections in dogs produce no effect; (2) if the rate of injection or the concentration of the drug is increased, there is a progressive increase in pulmonary blood pressure and a slow decrease in the systemic blood pressure; (3) large doses may precipitate death.

PATHOLOGY

Because of the diversity of types of reaction, there is no one set of pathologic findings which is found in all cases.

In those cases in which the prominent findings are in the brain, the term "hemorrhagic encephalitis" has been applied. Grossly the brain from such a case is congested and markedly edematous. Throughout the brain substance the vessels bleed easily from cut section. In the lateral wall and roof of the lateral ventricles are many punctate hemorrhages which may fuse into one hemorrhagic mass. In addition to this hemorrhagic ependymitis there are usually many small hemorrhages in the substance of the pons which if extensive enough may cause the pons to appear as one large hemorrhagic mass. On microscopic examination several findings are of importance. First, the brain substance is not degenerated, and it is readily seen that the large gross hemorrhage is made up of many small hemorrhagic areas. In many of the smaller blood vessels the lumen is occluded by a

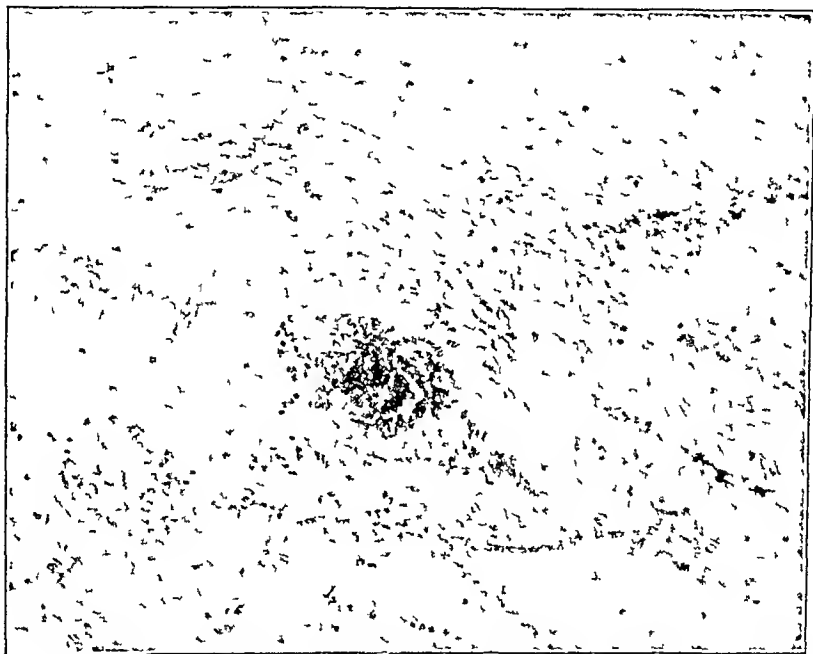


Fig. 2—Section of pons showing perivascular infiltration of cells and hyaline thrombosis of smaller vessels

hyaline thrombus. This hyaline thrombosis is not found in any other organ except the brain. In the present case the arterioles are surrounded by a collar of infiltrated wandering cells, mostly lymphocytes. In contrast to the accumulation of cells in the spaces of Virchow-Robin in other diseases, the infiltration in this case appears to be in the actual wall of the vessel and not in the perivascular lymphatics.

The pathologic changes in the liver in which jaundice is the prominent clinical symptom vary from a cloudy swelling to typical acute yellow atrophy depending upon the severity of the reaction. The relation of this hepatic damage to the injection of arsphenamine involves many questions. Is the hepatic damage due to syphilis, and its occurrence at this time only a coincidence? The weight of opinion would seem to be that the arsphenamine

itself causes the hepatic degeneration. The results of Wallis that injection of arsphenamine causes a slight to moderate hepatic insufficiency in cases where there are no clinical symptoms of liver disorder, would tend to confirm this opinion.

In most cases some pathologic condition of the kidney is reported. Various terms have been applied, such as 'tubular nephritis,' 'acute hemorrhagic nephritis,' etc., so that from a review of the reported cases it would appear that no definite and single change is characteristic. In the present case the nephritis was strictly tubular, involving all the tubules of the kidney and consisting of cloudy swelling and necrosis of the epithelium. As in the case of the liver changes, the etiology of the kidney pathology is obscure. Syphilis itself is capable of producing a mild nephritis and further simultaneous treatment with mercury must be considered. The ability of mercury to produce a nephritis is undoubted and in those fatal cases in which mercury and salvarsan have both been administered, the role of salvarsan in producing the nephritis must be carefully evaluated. In cases like the present one where clinically there was an anuria and at autopsy an acute tubular nephritis, with salvarsan the only drug given there can be little doubt that the salvarsan produced the nephritis.

The relation of salvarsan to degeneration of the adrenals is of interest. Experimentally it has been observed by Pearce and Brown and was observed in the present human case.

Other rarer pathologic lesions such as ulcerative enteritis and destruction of bone marrow,⁸ have been reported in isolated cases.

DISCUSSION

In looking over the results of the postmortem findings on human beings and the results of the experiments on animals, we find that there is a great similarity, indicating that by experiments on animals we may duplicate the conditions found in these unexplainable deaths from arsphenamine. Further, there is a striking resemblance of the pathologic findings in both the above two groups and the pathologic findings in an ordinary case of inorganic arsenic poisoning. The essential pathologic findings in all three classes of cases being

- 1 A destructive action on the endothelium resulting in congestion and hemorrhage in most of the organs of the body
- 2 The poor circulation resulting in edema of the brain and lungs in particular
- 3 A nephritis, usually of the tubular type but under certain circumstances taking on also a glomerular type
- 4 The finding of infarcted and necrotic areas in the various viscera in some cases
- 5 Necrosis of the liver, and sometimes acute yellow atrophy

From a critical survey of the pharmacology, proposed theories of pathogenesis, and the observed postmortem findings, and in view of the facts brought out above that arsenic poisoning, experimental arsphenamine poisoning and the fatalities with arsphenamine in human practice are very similar

in their pathology, we wish to cite what appears to us as the most likely theory in regard to the pathogenesis, namely,

1 That the immediate reaction, sometimes ending fatally, is due to the specific action of the arsphenamine molecule in its action by dilating the blood vessels of the body, resulting in some symptoms due to the local effects of congestion and stasis and others due to the same factors operating through the central nervous system centers. On this basis we may explain the "nitritoid crises," so-called because of their resemblance to the action of the nitrites in dilating the blood vessels, the headache due to a congestion of the brain, the vomiting and nausea due in part to the congestion of the stomach and to poor emulsion in the cerebral centers, and all the so called shock effects and vasomotor phenomena

2 That the essential nature of the delayed reactions is that of an acute or subacute arsenic poisoning due to faulty elimination of the arsphenamine molecule or the arsenic set free from it and the consequent liberation of the arsenic in inorganic form, which not being eliminated is retained and acts as a poison. The retained arsphenamine may act as a vasodilator and enhance the action of the arsenic as a capillary poison

3 Any part that adrenalin may play in the reaction, other than counter-acting the vasodilatation, is very doubtful in our mind

4 As to the embolic theory, although we cannot dispute it, at the same time we do not believe that it plays any essential part in the resulting pathology

In summary we may then say that the delayed arsphenamine reaction is nothing more than an arsenic poisoning due to the liberation of poisonous trivalent inorganic arsenic from the faultily eliminated arsphenamine

PROPHYLAXIS AND TREATMENT

It is not the purpose of this paper to enter into the field of the therapeutics of the organic arsenicals, but it is within our scope to point out facts which, gleaned from experimental pathology and human pathology, might be of value in the prevention of these fatalities

1 First the examination of the kidney is of prime importance since practically all the cases at postmortem show a nephritis. Therefore, it is of the greatest importance that in the administration of organic arsenicals that we do not give them to a patient whose kidneys are already having difficulty in eliminating the ordinary waste products of metabolism and do not overload them with the elimination of an inorganic metallic poison

2 Since it is known that mercury will act as a causative agent in producing nephritis, the use of any considerable quantity of mercury should not be practiced when the administration of arsenicals is occurring at the same time or within a short time. In other words, in the use of these two inorganic metallic poisons, we should use caution and occasionally give the patient a rest, so he may recover a normal metabolism

3 That the best care and technique which it is possible to use be practiced in the administration of the drugs, that is sterile, freshly distilled water, com-

plete neutralization if arsphenamine is used sufficient volume, not too fast injection, and not too heavy dosage at first or if the patient shows any intolerance for the drug

As to the treatment of these reactions, there is not a great deal of material or deduction of facts from human practice. It has been suggested by several authors that adrenalin given to the desired effect be used and several authors conclude that it is a specific. Jackson and Smith⁷ suggest the use of tyramine which differs in no great respect from adrenalin in its pharmacologic action.

It has been pointed out by Jackson and Smith, and rightly, that in the vasomotor and circulatory disturbance that follows the use of arsphenamine the heart is in a condition of instability and may go into delirium cordis. We know that adrenalin and the other amines may under certain circumstances produce delirium cordis therefore in the use of these drugs there is a certain danger which is unavoidable. This possibility is to be kept in mind but is not to be considered as a contraindication since the amines are the only drugs we have that offer any chance of benefit.

Since arsenic is found in the tissues of these patients and since arsenic poisoning is probably a big factor in these fatalities there is every reason for carrying out the usual procedures to promote the elimination of the poison by any means that the condition of the patient will tolerate.

CONCLUSION

In conclusion the following statement made in the British Report seems fitting: "There are and there always will be certain exceptional individuals who will react to the drug (arsphenamine) more severely than others and in whom a dose, or series of doses harmless to the average individual, may set up dangerous or even fatal complication."

Concerning the question of whether these fatalities are to be taken as evidence for the discontinuance of arsphenamine therapy the committee further states, "The committee has no doubt that in the interests of the patient himself, no less than in those of the community the choice should be in favor of arsenobenzol treatment. We believe that the very small number of deaths which are unavoidably due to this treatment are immeasurably outweighed by the deaths and disabilities which would arise if the older methods of treatment of syphilis were alone practiced. At the same time the facts which have been brought out in this report no less strongly emphasize the importance of the most scrupulous care in the administration of a drug which of necessity is employed in doses not far removed from the danger line."

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LABORATORY METHODS

A CYTOLOGIC KEY TO THE DIAGNOSIS AND PROGNOSIS OF NEOPLASMS*

By WM CARPENTER MACCARTY, M.D., ROCHESTER, MINN

EVERY pathologist has had some kind of an unwritten plan for grouping specimens upon which he feels he must pass clinical judgment. Usually he divides them into the three following divisions, each with two subdivisions: inflammatory (acute and chronic), neoplasms (benign and malignant), and questionable (inflammatory or neoplastic). The inflammatory group is characterized by one or more of the following phenomena: congestion, edema, necrosis, leucocytic lymphocytic and endothelocytic infiltration, fibroblastic and fibrocytic proliferation, hyalinization, and such cytologic changes as granular degeneration, fatty degeneration, vacuolization, pyknosis, and the presence of occasional giant cells. Certain of these differentiate the condition into acute and chronic inflammation although there is usually no sharp line of demarcation. The neoplastic group is characterized by the presence of a mass or masses of cells which do not have the exact histologic arrangement of normal tissues but seem to be displacing normal tissues by expansion or invasion. If the cells are regular in size and shape, are encapsulated, and have the morphology of normal adult types of cells, the condition is benign. If, on the contrary, the cells have not the low power arrangement of normal adult cells, are irregular in shape and size, and contain asymmetrical mitotic figures, are hyperchromatic, and replace normal tissues by invasion and infiltration, and especially if the mass is nonencapsulated, the condition is malignant. There seems to be, however, no sharp line of demarcation between these two, in some instances. The third or doubtful group is characterized by a combination of the characteristics of Groups I and II and as such presents the greatest differential diagnostic difficulties for all histopathologists.

These statements will call to the minds of many histopathologists the difficulties which they have experienced in conscientiously endeavoring to render clinical service in diagnosis and prognosis for the direction of therapeutic procedures. When the pathologist fails to make a diagnosis, the clinician must of necessity make a guess based on his own experience. When the clinician follows the pathologist's mistaken diagnosis and prognosis and directs his therapy accordingly, the pathologist, clinician, and patient usually suffer. Mistakes on the part of both pathologist and clinician are common and are due generally to several facts:

1. The clinical history of disease is not always differential unless the conditions are in the late or extreme stages. Any patient may have inflammatory and neoplastic conditions in the same lesion.

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, Washington, D. C., May 13, 14, and 16, 1927.

2 The gross appearances of disease are not differential in over 80 per cent of cases, and then only in advanced or extreme stages of the disease

3 Many diseases are internal and are therefore not macroscopically visible

4 There is no reliable serum chemical, physical or skin reaction which speaks for or against malignant neoplastic disease

5 Neoplastic diseases are coming to the profession as smaller and less visible lesions (Fig 1)

6 Detailed positive clinical diagnoses are becoming relatively less frequent

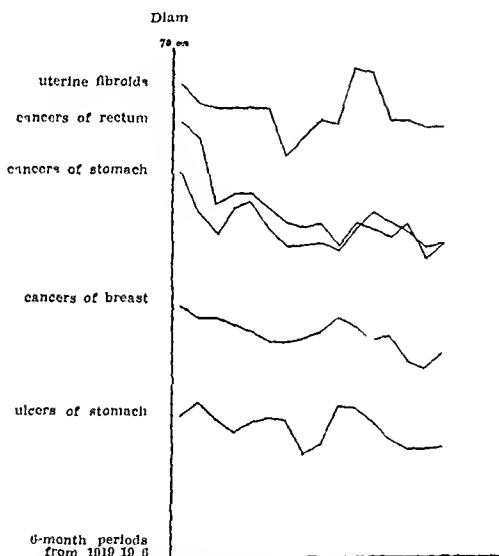


FIG. 1.—Diagram showing the decrease in size of cancers as the public and profession become educated to the necessity of early diagnosis

To these facts must be added lack of cooperation on the part of pathologists and clinicians and lack of interest in tissue pathology and clinical medicine on the part of most pathologists. In general it may be said that tissue pathology, in direct relation to the practice of medicine has been at a low ebb for the past twenty years and is just about to rise again. Part of the low ebb has been due to a failure on the part of pathologists to advance their own knowledge of human cytology. Pathologic histology has remained almost as it was given by Muller and Virchow fifty years ago; there have been some minor advances, but these have been along the lines of histology rather than cytology. There are at least three reasons for this: the limitation of

detailed cytologic study of postmortem fixed and embedded material, the pathologist's diversion into bacteriology, serology, immunology, and chemical pathology, and the lack of study of living and perfectly fresh human material

In the last twenty years a new opportunity for study has arisen through operative surgery. A new material has been presented, and few have taken the opportunity of making new observations and checking these against in

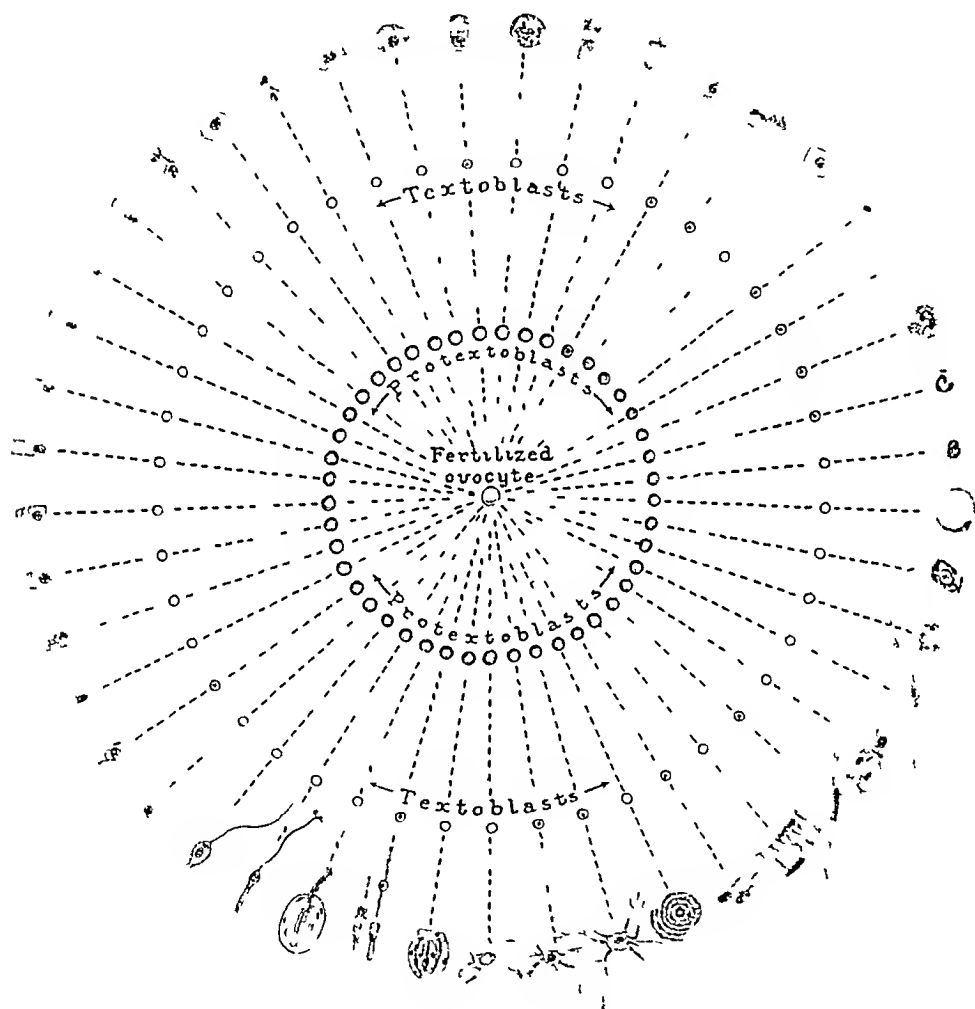


Fig 2—Diagram showing the evolution of at least forty-five different types of cells which constitute the tissues of the human body. No attempt is made to picture the exact morphology of each type although some of their characteristics are suggested.

timate clinical experience. So far as I know, the Mayo Clinic is the only place where this new opportunity has been given extensive practical attention over a long period of years. During the last twenty years more than 337,000 operations have been performed, and every operation was done with the pathologist present, all facts—the patient, history, visualization of the anatomic structural relationships, and the removed tissues—were available

and used by the pathologist in order to obtain data for correlation with macroscopic and microscopic findings

In 1905 Wilson described a method of frozen sections. It was intended primarily for diagnostic histologic purposes, but has been developed into a method for cytologic study with the highest powers of the microscope. Cells soon became the object of interest and study. At first, confirmation or refutation of clinical diagnosis was uppermost in the minds of both clinicians and pathologists. Later it became apparent that malignant conditions were discovered which were not suggested by or even suspected in, the clinical

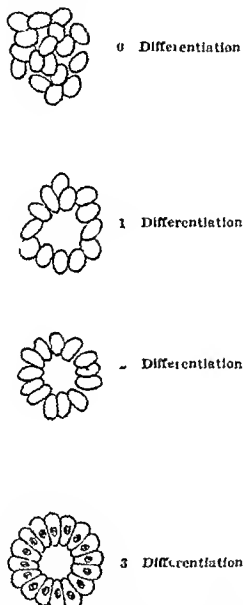


Fig. 3.—Diagrammatic representation of the recognizable stages of differentiation of a clonal unit during normal embryologic development. It also represents the stages of differentiation in neoplastic conditions.

history or gross appearances. As progress was made it was found that 57 per cent of all surgical cases presented material, and that 12.6 per cent of all cases required special pathologic consultation during operation, and that in 22 per cent of all surgical cases the diagnosis, prognosis, and therapeutic procedures were changed. In 0.5 per cent of all surgical cases, an unsuspected malignancy was found. The necessity for, and the value of, rapid and immediate diagnosis during operations brought about study and systematization of facts and the establishment of a plan for diagnosis and prognosis, only the cytologic part of which will be suggested at this time. It is

based upon the following facts the human body is an organism composed of groups of cells of at least forty-five different types, all of which have evolved from a fertilized human ovum (Fig 2) In this evolution, fertilization, segmentation, differentiation, and specialization occur Tissue differentiation, in the human body, occurs in such a manner that it may be divided into three recognizable stages (Fig 3) first, the establishment of the general alignment of the cells which is seen in the normal arrangement of adult tissue, the cells themselves remaining undifferentiated, second, the establishment of cellular polarity, such as is seen in fully differentiated tissue, and, third, the establishment of adult morphology of the cells In the condition

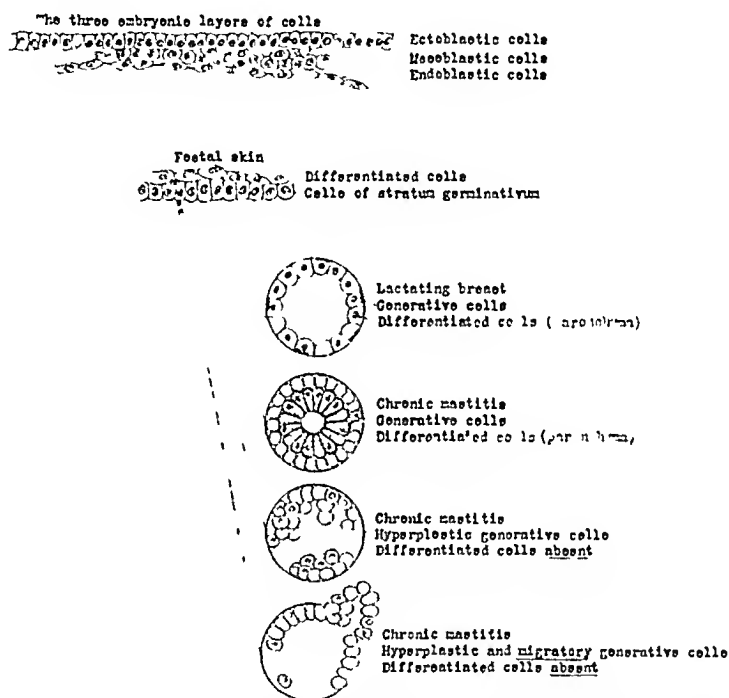


Fig 4—Diagrammatic representation of the evolution of a mammary acinus with its two types of cells (adenoblasts and adenocytes). The lower three diagrams show the three changes which are found in chronic mastitis. They represent hypertrophy, hyperplasia, and migration of adenoblasts. The migratory stage is malignant and the hyperplastic stage is potentially malignant.

of no differentiation, and the first and second stages, just described, the cells bear no morphologic resemblance to their adult forms.

During differentiation and specialization, nature provides for the phenomena of destruction and regeneration,—one a cause and the other an effect or reaction. Destruction of any tissue may be caused by many things. Regeneration of adult tissues occurs in two ways in the human body: directly (regeneration of adult cells from adult cells), and indirectly (regeneration from reserve cells). The malignant cell or the cell which has been called a "cancer cell," in which we are clinically especially interested, is evolved from the reserve cells although it may be derived directly from a cell which is normally regenerated directly. The study of the malignant cell is be-

havior and the natural defensive reactions of the organism to its abnormal proliferation and invasion of the rest of the body constitutes a problem of greatest practical economic importance, it is the key to both diagnosis and prognosis

Perhaps the simplest way to present the facts as they have been seen in the biologic and cytologic study of neoplasms is for us to confine our attention to principles in one organ. In Fig. 4 one sees diagrammatically illustrated, the evolution of the milk producing cells of the mammary gland. From the ectoblastic layer of the three layer stage of development of the

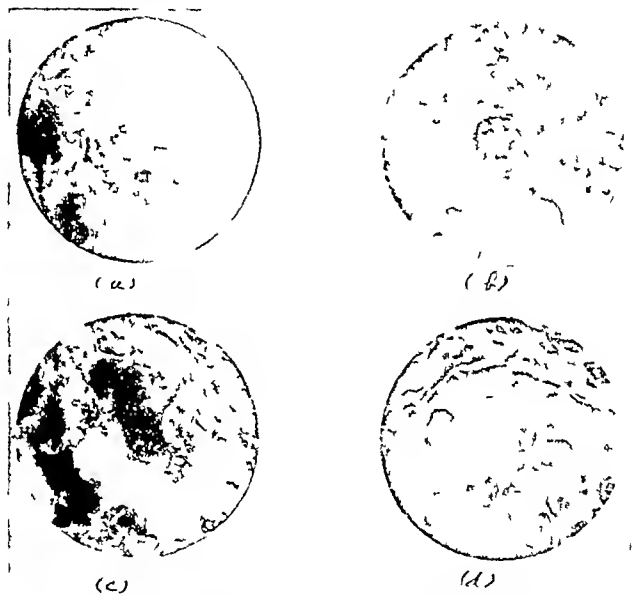


Fig. 3.—Photographs of malignant cells in a living state (a, b, c) and also in a fixed but not embedded state (d). The cells have positive diagnostic morphology.

embryo, the cells of the stratum germinativum of the fetal skin arise from them, arise the fetal squamous cells by differentiation. Also from them by multiplication, down growth into the subepithelial tissues and differentiation the lining cells of the mammary tubules and acini arise. It may be seen that there are two layers of cells in each normal mammary acinus and tubule. The cells lying adjacent to the lumen are secretory (adenocytes) and those lying next to the stroma are the reserve cells (adenoblasts). In chronic mastitis some one or more unknown things or conditions destroy the secretory cells, the reserve cells become hypertrophic or enlarged. This is a common picture in chronic mastitis with or without the presence of can-

cell. In some of the acini the reserve cells not only become hypertrophied, under the condition of destruction of the secretory cells, but also become hyperplastic, they increase in number and partially or completely fill the acinus. In this condition the cells are undifferentiated, they have larger nucleoli and nuclei than do adult or differentiated cells. The undifferentiated cells are frequently indistinguishable from those of cancer despite the fact that they are still within the confines of the acinus. In some acini, in some chronic mastitides in which this proliferative condition exists, one finds the line of demarcation between acinus and stroma destroyed by the migration

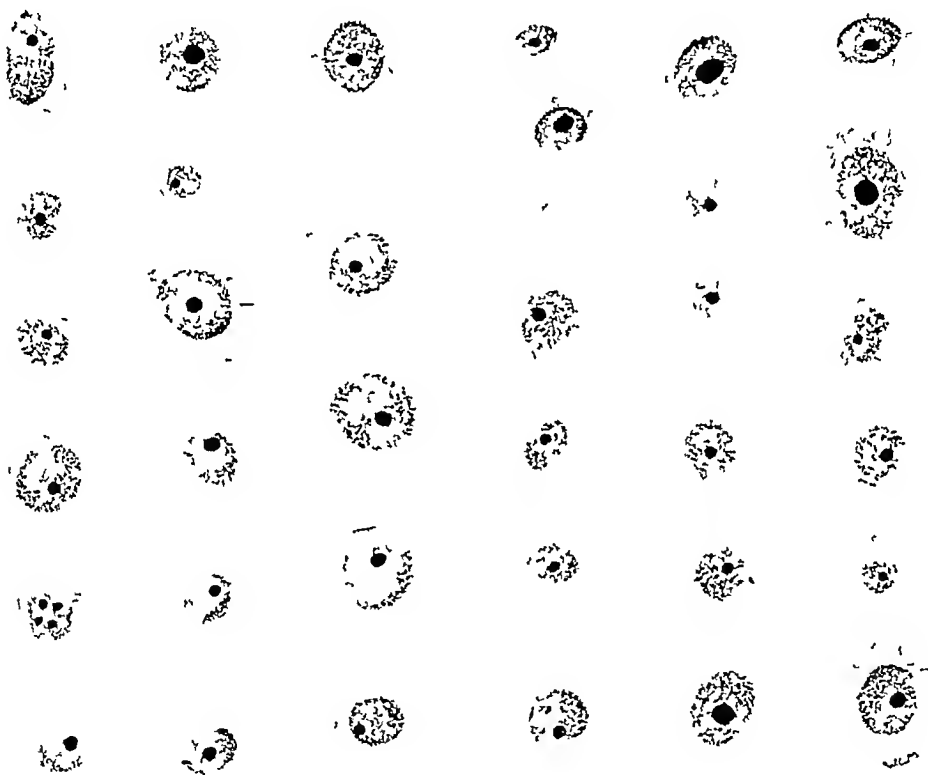


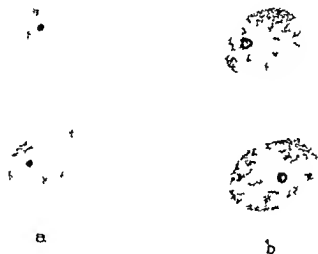
FIG. 6—Sketches to scale of malignant cells from many sources. They represent those for carcinoma, sarcoma, lymphosarcoma, lymphatic leucemia, and malignant endothelioma. It is impossible to differentiate one from the other, but each one has a morphology unlike any normal regenerative or adult cell in the human body.

of undifferentiated cells. The last picture is the one we call cancer, the cells are of the type seen in Figs 5 and 6.

The question arises: Has the malignant cell a morphology by which it may be recognized? Textbooks describe the cancer cell as having an irregular shape with an irregularly shaped nucleus which takes the stains densely and frequently shows an asymmetrical mitotic figure. They rarely, if ever, say anything about the nucleolus. This description applies to those cells in pathologic tissues which have been dead for some time, have undergone cytologic changes coincident to and following death, and have been fixed in strong solutions and then embedded in celloidin or paraffin. It is not the

picture which one sees in living tissues or tissues which, although dead, have just died and are studied in an unfixed condition with oil lenses. Under these less destructive conditions the cancer cell is an ovoidal or spheroidal body with no irregularities of "cell wall," nucleus or nucleolus, the demarcations of the component parts of the cell are perfectly sharp and distinct, the granules of the cytoplasm and nucleoplasm are fairly uniform in size, the mitotic figures are sometimes multipolar and they are never asymmetrical and irregular in my experience. The whole cell when studied in the fresh condition is the object of study, it is not cut in planes. Its constituents are not coagulated and are, therefore transparent or translucent. There is no necessity for thin sections such as one attempts to obtain with eeloidin and paraffin methods.

The cancer cell may perhaps not always be distinguished from a normal regenerating cell but this can be done frequently because there is a difference in volume relationship between nucleolus, nucleus and the whole



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Fig 7—Diagrammatic sketches of reparative regenerative cells and malignant regenerative cells to show some of the differences in morphology. The reparative cells are more delicate have smaller granules and smaller nucleoli.

cell in the reparative regenerative cells and malignant regenerative cells. The nucleolar nuclear area ratio is from approximately 1 to 23 in malignant cells and 1 to 50 or more in reparative regenerative cells. There is also a difference in the density of the nucleoplasm and cytoplasm in the extreme exemplars of these two cells. The regenerative cell is more delicately constructed (Fig 7), and its nuclear granules are usually finer, hence, the nuclear outlines are more delicate. These are some of the differential points. There are qualities which words cannot describe. One learns to recognize the types of cells from experience with actual clinical proof of diagnosis and prognosis just as one recognizes members of his family and friends. It is not always possible to describe our friends in such a manner that others can recognize them. Expertness in the differential recognition of malignant cells, reparative regenerative cells, and adult cells comes through constant contact with them, checked by clinical proof of diagnosis. The problem is one of cytology, not histology. The malignant cells which have just been described are

malignant because they invade the surrounding tissues, spread to distant parts of the body where they multiply independently, disturb the vitality of the whole organism directly and indirectly, and eventually cause death.

One of the greatest problems of the clinicians and pathologists is prognosis, and data upon which it may be made have been largely compiled from statistics based on the average length of life from the time the physician or surgeon sees the patient. At best it is based upon impressions and some personal opinions since there are so many variables and unknown factors in the problem. In general, one considers the following factors which seemingly, if not definitely, influence the relation of new growth to longevity: size of growth, cellular character of growth, age of the host, duration of the lesion, relation of growth to nutrition, emaciation in relation to food intake, proximity of growth to vital structures, lymph-glandular involvement (regional and distant), distant organic metastases, multiplicity of the lesions, character of previous treatment, and the morale of the patient.

The influence of these factors is purely relative and very general, and we find, in practice, failures when either good or bad prognoses are given. Recognition of the possibility of prognostic incorrectness has led many wise physicians to give guarded prognoses, or they merely attempt, in diplomatic manner, to improve the morale of the patient and very frankly warn the relatives. This humanitarian and necessary habit of our profession, great and noble as it is, should not prevent us from a strenuous search for facts which lend efficiency to practice since, in this day of economic expertness, the human individual wants to know, frequently, the time left for the completion of his plans of life.

The facts, presented here, are the result of unsatisfactory experience with the lack of definiteness in prognosis. Thus, patients with large cancers of the stomach (or some other organ) with complete regional lymph-glandular involvement have been given the worst prognosis routinely, and those with small lesions and no glandular involvement a better prognosis. While this method of consideration has been good in general, there have been many instances of unexpected and quite surprising variations from the general rule, some patients with small lesions and no apparent glandular involvement die of recurrence very rapidly, others, with large and extensive lesions, live long—sometimes ten and fifteen years—after local removal.

Besides the factors which have been mentioned as being possible aids in formulating prognosis, there are others which can be and have been studied. They are, however, of value only after removal of the neoplasm since they are microscopic in character. The four factors which have thus far been studied are *lymphocytic infiltration*, *fibrosis*, *hyalinization*, and *cellular differentiation*, the last factor being merely an index of the favorableness under which the invading cells are living. By lymphocytic infiltration is meant that infiltration which is in immediate contact with the cancer cells, and this intimate contact of reaction is also essential in determining the relation of the reactions of fibrosis and hyalinization. These three factors are considered in sequence, lymphocytic infiltration first, fibrosis and hyalinization, third. By cellular differentiation is

cellular modification coincident with special function. There are degrees of this differentiation recognizable in the normal evolution of specific tissues and these stages are frequently seen in the study of neoplastic cells (Fig. 3). Thus, the cells of a so called adenocarcinoma or a squamous celled epithelioma are in one of two stages of partial differentiation. Biologists have recognized that the power of growth is indirectly proportional to the height of the differentiation, and this law holds good very apparently for the cells of neoplasms. These four factors are visible and in the work presented here, have been studied and recorded without any knowledge of the actual length of life in each case. After the observations in the neoplasms had been recorded, they were assembled with the facts pertaining to postoperative life.

There are several facts of scientific interest and practical importance visible in these observations.

Whenever the factors are present the average length of life is greater than when they are absent. This is true in all four groups of cases studied.

When all of the factors are present the average length of life is much greater than when all of the factors are absent.

The variations of frequency of the presence of the different factors in different organs is of interest and suggests that the defensive mechanism while a general phenomenon acts differently in different regions of the organism.

To this series of observations may be added the results found by Broders in the study of epithelioma. Based upon the degrees of cellular differentiation described by the writer he has very successfully attempted prognosis and divided the epitheliomas into four clinical groups. Group I being the most favorable and Group IV being the least favorable. Despite the fact that his clinical group numbers are in reverse order of the actual stages of differentiation in relation to rapidity of growth the facts of growth relationship are according to the biologic law. His numbers I, II, III, and IV represent clinical groups and not the order of degrees of differentiation (0, 1°, 2°, 3°).

That these are all of the defensive factors in the organism is not to be expected, but that they are all factors which form a part of any key to prognosis can hardly be questioned from the uniformity of the results of observation.

It may be correctly said that the Master Key to diagnosis of malignant and benign neoplastic conditions and inflammatory conditions and prognosis is a checked experience with the differential detailed morphologic characteristics of adult tissue cells, reparative regenerative cells and neoplastic cells. All of these may be recognized in the fresh unfixed condition with or without stain if the oil lens of the microscope be used. Freezing methods and the different staining methods are only means of decreasing time of search. They, in themselves, will not make great and reliable diagnosticians or prognosticians any more than a good box of colors a piece of canvas, some good brushes and an easel will make an artist. I mention this because more emphasis has been laid by writers and talkers on the method than on the necessary training and experience. For the benefit of those who are

always hunting short cuts, I must say, after long and constant experience and study, that there is no short cut to becoming a great clinical pathologist. The subject cannot be learned from books any more than the art of literature or painting can be learned from books. It is the short-cut pathologists who have thrown discredit upon tissue pathology which in truth is, after all, the basis of our knowledge of all disease. May I say also that no one individual can possibly be efficient in tissue pathology, bacteriology, serology, immunology, radiology, roentgenology, chemical pathology, parasitology, and general clinical microscopy. And still this is what is expected by the great majority of hospitals. They even expect it from young men and women. It cannot be done, and it should be discouraged by all members of our profession. The differentiation of pathologic conditions and then clinical interpre-

	STOMACH 99 CASES years	BREAST 92 CASES years	RECTUM 102 CASES years	SKIN 29 CASES days
Average length of postoperative life with differentiation -----	2 73	3 67	1 54	534 1
Average length of postoperative life without differentiation -----	2 56	2 57	1 08	274 7
Average length of postoperative life with lymphocytic infiltration -----	2 73	2 51	1 57	496 2
Average length of postoperative life without lymphocytic infiltration -----	2 7	2 48	1 31	346 6
Average length of postoperative life with fibrosis -----		2 72	1 53	655 7
Average length of postoperative life without fibrosis -----		1 87	1 29	295 6
Average length of postoperative life with hyalinization -----		2 81	2 33	449 6
Average length of postoperative life without hyalinization -----		2 21	1 44	437 9
Average length of postoperative life with differentiation and lymphocytic infiltration --	2 8	3 78	1 59	644 5
Average length of postoperative life without differentiation and lymphocytic infiltration --	1 55	2 45	0 71	204 0
Average length of postoperative life with differentiation and fibrosis -----		3 87	1 58	808 3
Average length of postoperative life without differentiation and fibrosis -----		1 96	1 15	257 5
Average length of postoperative life with differentiation and hyalinization -----		4 0	2 33	587 0
Average length of postoperative life without differentiation and hyalinization -----		2 04	0 61	257 5
Average length of postoperative life with lymphocytic infiltration and fibrosis -----		2 69	1 65	739 8
Average length of postoperative life without lymphocytic infiltration and fibrosis -----		1 4	1 17	155 2
Average length of postoperative life with lymphocytic infiltration and hyalinization -----		2 76	2 25	404 0
Average length of postoperative life without lymphocytic infiltration and hyalinization --		1 68	1 27	255 5
Average length of postoperative life with fibrosis and hyalinization -----		2 89	2 33	453 8
Average length of postoperative life without fibrosis and hyalinization -----		2 05	1 28	282 5
Average length of postoperative life with lymphocytic infiltration, differentiation, fibrosis, and hyalinization -----		4 4	2 25	444 6
Average length of postoperative life without lymphocytic infiltration, differentiation, fibrosis, and hyalinization -----		1 52	0 76	54 0

tation in the light of the best interests of the patient is an art. Like any art it involves knowledge of technical details, experience, and a broad conception of disease.

DISCUSSION

Dr B. T. Terry—It has been my pleasure to work in Dr. MacCarty's laboratories since June 14, 1926, and many times I have seen him make diagnoses of malignancy either from one cell or from a very small number of cells. To do this, however, very great experience is necessary. In spite of the fact that I have tried conscientiously for nearly a year to learn the criteria Dr. MacCarty uses, I am not yet sure of malignancy from the study of one cell only. An example may be worth while. Not long ago a freshly removed lymph node was brought for diagnosis. In the first frozen section one cell was found which Dr. MacCarty regarded as malignant and at once reported it as such to the operating room. Dr. MacCarty pointed out the cell to me. I studied it carefully and thought I saw what he did but, lacking his experience, I could not be sure of malignancy from this cell alone. Other sections of the same node were then cut and studied and in some of these there were large enough groups of cells for me also to be sure. As the cytologic diagnosis of malignancy now requires an enormous amount of experience, I am hoping that Dr. MacCarty will continue his work and that he will be able so to simplify his criteria that he can save us the labor of learning forty-five different cells. If he can teach us a simple, sure, and easy way of recognizing malignancy by the study of a single cell, the entire world should be grateful to him.

DIFFERENTIAL BLOOD COUNTS

A COMPARISON OF THE ACCURACY OBTAINED BY VARIOUS METHODS*†

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A DIFFERENTIAL blood count is one of the most common and also one of the most important of routine laboratory procedures. The question has no doubt arisen in the minds of many as to the accuracy of these counts since authors of various texts on clinical laboratory methods do not agree as to which method of making a blood film gives the most satisfactory spread for such work. In his recent book Haden¹ says, "Blood films may be made either on cover glasses or on slides. For careful work, preparations on cover glasses are far preferable. For the accurate differential counting of the leucocytes such preparations are essential." Cummer² prefers the use of cover-slips over slides because in his opinion the leucocytes are unevenly distributed in the latter case. Other writers, Kilduffe³ and Simon,⁴ prefer the use of slides over cover glasses for the preparation of blood films.

During the summer of 1926 a questionnaire was prepared and sent to various laboratory directors. Among the questions asked were those pertaining to the method used in making the blood film, the part or parts of the film counted, and the number of cells routinely classified for a differential blood count. While only eighty-two answers were received, they probably give a fairly good idea as to the methods used in this country. Seventy-five workers use slides in the preparation of blood films, while only seven use cover-slips. Of this majority about 60 per cent place the drop behind the spreader slide, while about 30 per cent have the drop ahead and either push or drag the cells across. The remaining 10 per cent is composed of those who use the cigarette paper method or who have special methods which in general may be considered as a combination of the first two mentioned.

It was learned that fifteen of the laboratories replying classify only one hundred cells for a differential count. Twenty-three observed two hundred cells, three, three hundred, and three five hundred, while the remainder, which comprises about 50 per cent, classify from one hundred to one thousand cells, depending upon the accuracy desired. A majority of this latter group, however, count not more than three hundred leucocytes.

Since there is a difference of opinion as to the best method of preparing a blood film for differential counting, and also a difference as to the number of cells which should be classified, an attempt was made to see, (1) if one method was more accurate than another, and (2) to learn how many cells should be observed for an accurate differential count. In order to arrive at

*From the Department of Clinical Pathology, University of Colorado School of Medicine.

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the conclusions given, more than two hundred thousand leucocytes were studied

In general the first question was attacked in the following manner. Two preparations were made, one by a method known to be accurate when every leucocyte was counted and the other by one of the methods which was to be tested.

Since none of the slide methods were known to be absolutely accurate, due to the fact that certain cells might adhere to the spreader slide, the cover slip method was tried. But here it was learned that films made in this manner were usually unsatisfactory either because of ruptured cells or because of areas in which the erythrocytes were so thick that the leucocytes could not be classified.

Fairly satisfactory films were usually obtained by using two slides in a manner similar to the two cover method but too often the preparations were not good, owing to differences in curvature of the glass.

An accurate method was then developed which was found to be very satisfactory and which may be called 'The Slide and Cover Method'. The technique is very simple, and it is practically impossible to obtain a film in which all of the cells cannot be counted provided that certain simple rules of technique are followed. A small drop of blood is placed near the end of a clean slide, and a medium to thin cover slip is dropped on the blood which will then flow out in a thin layer if the glass is clean and if there is not too much curvature to the slide. If the film does not flow out properly, very gentle pressure will bring this about. As soon as the blood has stopped flowing, the slide is held on the table with the left hand and the first two fingers of the right are placed near the edges close to the left end of the cover glass. Then with an even, fairly rapid pull it is slid along the slide in the direction of its long axis. The amount of pressure necessary is only enough to keep the fingers from slipping from the spreader. This leaves a smooth even film covering a large or small area of the slide depending upon the size of the drop used. It is interesting to note at this point that only very rarely are any cells found on the cover slip and when this does occur the differential percentage of leucocytes is the same as that found on the slide. The use of two fingers in sliding off the cover is very necessary for two reasons. If a convex slide is being used a finger on each side of the glass will cause it to conform better with the curvature of the slide and thus allow a more uniform film. If only one finger is used, there is a tendency for it to slip off. When there is a second attempt at removal, it will be found that the adhesion is so great that many of the polymorphonuclear leucocytes will be ruptured, a condition very commonly observed in cover slip preparations.

After developing a method known to be accurate certain observations were made in order to be sure that the results had been properly controlled.

It was found by trial that it was not necessary to observe every leucocyte on a slide in order to obtain an accurate differential count. Several slides were prepared by the slide and cover method every cell counted and the true percentage of polymorphonuclear leucocytes was found. Then each slide was recounted classifying the cells in every other row across each film the

edges of which were always included. In every case it was found that the neutrophilic percentage checked within 2 per cent of that found in the first instance. Consequently, all of the counts recorded in this paper represent the leucocytes found in every second row across each film.

A skin puncture was made and four films prepared at minute intervals from each of two normal individuals. A great deal of milking of the finger was necessary in each case in order to obtain enough blood for the fourth smear. When every second row of each preparation was counted, the differences between the highest and lowest polymorphonuclear findings were less than 15 per cent.

Although we know that whenever the skin is injured there is a gathering of certain leucocytes at that point of injury, this occurrence either is not rapid enough or not marked enough to make any difference in the blood picture as it is obtained routinely. Also since in both cases squeezing the finger was necessary in order to obtain blood for the fourth film, that fact makes no difference in the results of a differential blood count, although we know that it does cause an error in total counts.

Neither does the size of the drop used have any effect. Small films, having less than two thousand leucocytes, were made, and then some were made having more than seven thousand. The results obtained in these cases always corresponded within 2 per cent.

Having answered some necessary questions, the accuracy of the more commonly used methods of preparing blood films was checked in the following manner. The finger was punctured in the usual way, the first drop of blood wiped off, and a smear was made on a slide using the above known accurate slide and cover method. Then one or more films were prepared from the same puncture by the method which was being tested for accuracy.

The four methods tested in this manner were (1) *The drop behind the spreader method*. This, in brief, consists of placing the drop of blood behind

TABLE I

ACCURACY OF RESULTS WHEN BLOOD IS PLACED BEHIND SPREADER POLYMORPHONUCLEAR
PERCENTAGE

(every second row across each film counted)

PATIENT	SLIDE AND COVER METHOD	DROP BEHIND SPREADER ALL BLOOD USED	REMARKS
M O	60.5	61.3	Many cells at end
F D	55.0	54.5	Many cells at end
W B	66.3	65.1	Many cells at end
ALL BLOOD NOT USED			
F D	54.4	53.1	Thick film
M P	62.3	62.6	Thick film
F D	51.7	50.1	Thick film
L K	66.8	60.2	Medium film
M O	63.6	61.9	Medium film
F D	51.9	43.5	Thin film
L P	67.5	51.4	Thin film
E M	55.4	49.0	Thin film
H H	58.1	49.8	Thin film
L P	63.8	58.4	Thin film

the spreader slide which is held at an angle of about 40° . This is then pushed forward, and the blood follows leaving a smooth, even preparation. (2) *The dragging method*, somewhat similar to the first, except for the fact that the drop is placed in the acute angle in front of the spreader slide and the cells dragged across. (3) *The pushing method* having the drop of blood placed in the obtuse angle in front of the spreader which is then pushed forward across the slide. (4) *The cigarette paper method*. These films are prepared in the manner described for the dragging method, except for the fact that a cigarette paper is used for a spreader instead of a slide.

The results of the accuracy of these methods are shown in Tables I, II,

TABLE II

ACCURACY OF RESULTS OBTAINED BY DRAGGING METHOD. POLYMORPHONUCLEAR PERCENTAGE
(Every second row across each film counted)

METHOD	PATIENT	REMARKS	P	M	N	%
Slide and Cover Dragging	F D					52.7
		All blood used				52.1
		All blood not used				52.9
		All blood not used				55.4
Slide and Cover Dragging	H H					55.2
		All blood used				56.5
		All blood not used				54.2
		All blood not used				56.0
Slide and Cover Dragging	M P					60.3
		All blood used				61.9
		All blood not used				68.0
		All blood not used				66.9

TABLE III

ACCURACY OF RESULTS OBTAINED BY PUSHING METHOD. POLYMORPHONUCLEAR PERCENTAGE
(Every second row across each film counted)

METHOD	PATIENT	REMARKS	P	M	N	%
Slide and Cover Pushing	F D					57.7
		All blood used				57.1
		All blood not used				48.4
		All blood not used				47.8
Slide and Cover Pushing	F J					56.5
		All blood used				55.8
		All blood not used				49.7
		All blood not used				55.2 (thick film)
		All blood not used				51.8
		All blood not used				54.1
		All blood not used				51.3

TABLE IV

ACCURACY OF RESULTS OBTAINED BY CIGARETTE PAPER METHOD. POLYMORPHONUCLEAR PERCENTAGE
(Every second row across each film counted)

PATIENT	SLIDE AND COVER METHOD	CIGARETTE PAPER METHOD
L P	61.4	62.7
M P	57.9	56.6
F D	63.9	64.2
W B	66.1	65.4

III, and IV, the figures in which represent the polymorphonuclear percentages obtained when all of the leucocytes in every second row across each film had been classified in groups of one hundred cells

DISCUSSION OF RESULTS

Table I shows the results obtained when the film was made by placing the drop behind the spreader slide and the findings compared to those of the slide and cover method. It will be noted in the first three counts that when all of the blood was used in making the film, the results checked very closely with the accurate findings, thus showing that certain leucocytes do not adhere to the spreader, and that a differential count made from this kind of smear is accurate provided enough cells are classified. In each of these three slides many leucocytes were found at the extreme end of the film. This fact may be seen in practically every film which is made by means of a spreader slide, no matter what special kind of technique is used. Wright explains this by stating that the plasma, red corpuscles and the smaller leucocytes are deposited on the slide first, and the larger leucocytes are pushed or dragged ahead of the spreader until all of the fluid has drained away, leaving these cells stranded. This observation will explain very nicely the results obtained in the latter part of the table. It will be seen that in the last ten counts all of the blood was not used in preparing the film. Some of the films were thick, some medium, and some thin, and the thinner the film the lower the percentage of neutrophils. When a thin film is made, there is a lessened number of small cells deposited, and consequently a large number of large cells are floated to the end, and when all of the blood is not used in preparing the slide, these cells are not found. When a thick film is made, a greater number of small cells and consequently a greater number of large cells adhere, thus giving a more accurate differential count. At this point it is well to explain what is meant by a thick and thin preparation. Those in which the red corpuscles are well separated are considered thin films, those in which the red cells overlap to some extent are called medium ones, and the thick films are those in which the cells are piled up to such an extent that rouleaux formation may be noted.

Table II shows the accuracy of counts made on films prepared by the dragging method. Here again the findings show that cells do not adhere to the spreader slide in such amounts as to interfere with correct results. But contrary to the findings in the first method tested, the percentage of polymorphonuclear leucocytes is not decreased, but usually slightly increased above the correct findings when all of the blood is not used in making the preparation, due possibly to the fact that rather thick films were made in each case.

In Table III it may be observed that when all of the blood is not utilized in preparing the films, the polymorphonuclear percentage is lowered. But even in very thin films this decrease is not as marked as in those prepared with the drop behind the spreader, although it is more pronounced than when the dragging method is used. When films are prepared by this pushing method, some of the blood will be seen to flow under the spreader slide and

because of this fact the film is really prepared by pushing some of the cells ahead and allowing some to follow. Since this method may be considered as a combination of the two preceding ones discussed, the results are those which might be expected when all of the blood is not utilized in preparing the slide.

The cigarette paper method is an accurate one as shown in Table IV. Its chief disadvantage is that many neutrophils are found at the edges and at

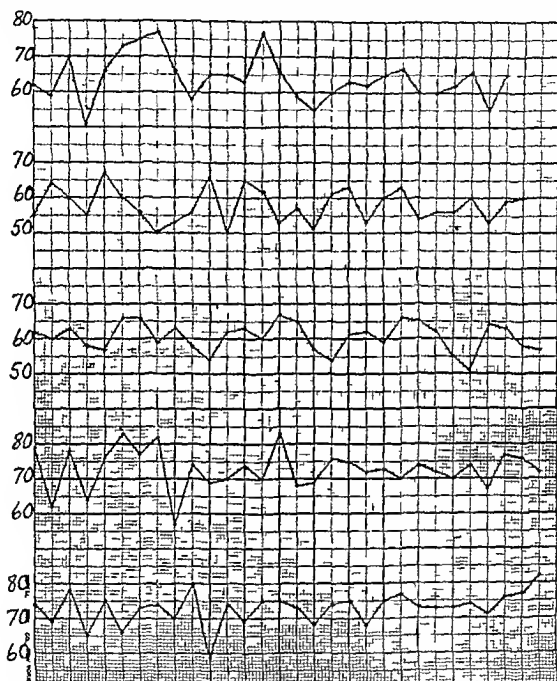


Chart 1.—Percentage graphs of neutrophilic content of five cover slip preparations in consecutive counts of 100 leucocytes (Every second row across each film counted.)

the end of the film because they have been floated out as the cigarette paper has been pulled across the slide.

Films made by the cover slip method were then tested for accuracy, and the results showed that preparations of this kind were satisfactory for differential counting. However, I cannot agree with those who say the leucocytes are more evenly distributed on cover slips than on slides. A large number of cover slip films were counted in groups of one hundred cells and the results brought out the point that there was as great a variation in the neutrophilic percentage as there was in slides prepared by any of the methods.

in common use. If one counts every leucocyte on both covers, he will always find a variation of at least 15 per cent between the highest and lowest polymorphonuclear percentage. He will not find this difference to be any greater in films prepared on slides if the proper kind of technique has been employed.

Much better technique is required to make satisfactory cover-slip smears than those made on slides. When I began this work, I was unable to obtain constantly what I considered to be a good cover slip preparation. I thought that the variation in percentage obtained might be due to the fact that my films were not as smooth as they should be. I then wrote letters to the various workers who had said in the questionnaire that they were using cover-slip preparations for differential counting, telling them of this difficulty, and asked them to send what they considered to be a satisfactory film. Several specimens were obtained which were counted, and the variation was found to be as great as in the previously counted ones. The results of a few representative counts are shown in graphic form in Chart I.

Because of the time required to count every cell or even half of the cells on a slide or cover, it is not practical to use such a method in routine work. The true percentage of cells was obtained from each slide when the first part of the work had been done and an attempt was made to learn whether or not a fairly accurate percentage could be obtained by counting three groups of one hundred cells each on different parts of the film. Two of these counts were made on each film, and the results were compared with those obtained when every second row had been examined. The first count was made in the following manner: One hundred leucocytes were classified in each of three areas extending across the film, one near the beginning, one at the middle, and one near the end of the preparation and the average of the three counts obtained. The other count was made by examining three areas along the long

TABLE V

ACCURACY OF RESULTS WHEN 300 CELLS ARE CLASSIFIED POLYMORPHONUCLEAR PERCENTAGE

METHOD USED	PER CENT WHEN EVERY SECOND ROW HAD BEEN COUNTED	PER CENT WHEN 300 CELLS HAD BEEN COUNTED THROUGH SHORT AXIS OF FILM	PER CENT WHEN 300 CELLS HAD BEEN COUNTED THROUGH LONG AXIS OF FILM
Slide and Cover	58.4	61.0	63.3
Slide and Cover	61.3	60.7	57.0
Slide and Cover	60.8	61.0	57.3
Slide and Cover	54.4	54.3	55.7
Slide and Cover	53.1	55.0	55.7
Slide and Cover	51.7	50.3	48.3
Slide and Cover	51.4	62.3	62.0
Slide and Cover	57.9	55.3	55.7
Slide and Cover	63.9	62.0	65.0
Slide and Cover	60.3	58.0	58.7
Slide and Cover	57.7	56.3	59.3
Drop behind spreader	50.1	55.7	49.3
Drop behind spreader	60.2	60.3	57.0
Cigarette paper	65.7	58.7	65.3
Cigarette paper	56.6	53.7	57.3
Cigarette paper	64.2	58.0	64.7
Cigarette paper	68.0	65.0	66.0
Dragging	61.9	61.0	59.0
Dragging	66.3	64.0	63.0
Dragging	55.2	52.3	55.0
Pushing			

TABLE 11
SHOWING HIGHEST AND LOWEST PERCENTAGE IN FIVE PUNGS MADE BY VARIOUS METHODS WHEN FIFTY SPIND ROW HAD BEEN COUNTED

METHOD	HIGH	LOW	DIFF	HIGH	LOW	DIFF	HIGH	LOW	DIFF	HIGH	LOW	DIFF	AVE.
Slide and Cover	67	51	16	65	54	11	68	54	14	64	53	11	124
Drop behind spreader	73	52	21	75	51	24	73	53	20	72	50	22	204
Dragging	78	61	17	79	52	17	73	57	16	64	50	14	182
Pushing	61	47	14	58	42	16	71	46	25	64	41	23	192
Cigarette paper	74	60	14	78	48	30	70	43	27	70	60	10	182
Cover slip	77	51	26	67	50	17	67	51	16	83	57	26	216

axis of the slide. The results of some of these counts may be seen in Table V which represents differential counts from various methods of preparing the film.

It will be noted that an exact count is seldom obtained when only three hundred leucocytes are studied, but since most of the counts do not differ more than 3 per cent, it makes little difference from a clinical standpoint. This slight error will be found only if good technique has been used in preparing the film and the proper areas counted. It is maintained by some that great numbers of cells are found at the edges of a smear which has been prepared by a two slide method. But a condition such as this is seen only when the technique has not been good. When working with any two slide method, if the film is begun before the drop has completely flowed along the spreader slide, many polymorphonuclear leucocytes will be found along the edges. The same thing will occur if too large a drop has been used. It is true that large numbers of cells are found near the end of a film prepared on a slide by the usual methods when all of the blood has been employed in the preparation. This will cause an error in differential counting unless one of the three groups classified has been obtained from this area. If this third part of the film has not been considered, the neutrophilic percentage will be somewhat lower than it should be.

Because of the fact that many blood films are not prepared or counted in the proper manner, it is undoubtedly true that the slide and cover method holds several advantages over others. The technique is simple. Ruptured cells in excess may be found, but this is due to the fact that either too great pressure has been placed on the cover or it has not been removed with an even, fairly rapid movement. Even when ruptured cells are found, the number is not as great as that observed in many cover-slip preparations. The leucocytes are more evenly distributed than in other methods as may be seen in Table VI. Large numbers of cells are not found either at the edges or at the end of the film. When every cell on a slide is classified, one will not see as great variation in percentages as is usually observed in the more commonly used methods. But even though the cells are more evenly distributed, one cannot be sure that he is accurate within 10 per cent when only one hundred cells have been observed. The method utilizes all of the cells in the drop of blood, and so one can be sure that none of the large leucocytes have been floated out to the end and allowed to dry in an area too thick to be counted.

CONCLUSIONS

Blood films properly prepared on slides give an accurate differential count provided enough leucocytes have been classified and all of the blood has been utilized in making the preparation.

If all of the blood in the drop has not been used in making the film, the neutrophilic percentage will be decreased proportionately to the thinness of the smear.

A differential blood count on a film prepared by any of the methods previously discussed is seldom accurate within 10 per cent if only one hundred cells are classified.

A differential count made from either slides or cover slips is accurate within 3 per cent if correct technique has been employed in preparing the film and if three hundred cells in three different areas have been studied.

Leucocytes in cover slip preparations are not more evenly distributed than in those made on slides.

A method for the preparation of blood films is presented which is simple, accurate, and gives a more even distribution of leucocytes than is obtained by other methods.

I wish to thank Dr James C Todd and Dr F P Mudge for valuable suggestions and criticisms during the progress of this work and also Mrs Frances Dunning for technical assistance.

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- ⁵Wright, Sir A E Technique of the Test and Capillary Glass Tube ed 1 London 1912 Constable and Company Ltd p 6

DISCUSSION

Dr A H Sanford—I was told of this method by Dr Todd a few months ago and was pleased to find out how simple it was. I believe that those of you who are teachers of clinical pathology will find it worth while to demonstrate this method to your students and observe the results of its use in their hands.

Dr Robert A Keady—I would like to ask one question. Is this to be an actual routine for one who has to count 1, 20 or 30 differential counts in a day using three hundred fields to each slide? I would like to ask the reaction to this question.

Dr F B Johnson—It is a problem to decide the most accurate way to do these counts. I realize more than you would otherwise this error in dealing with my students. This is a new method and I will be glad to try it out. I have been trying out a method in making differential counts using the same dilution that we make our total counts from. The disadvantages are that it is very hard to differentiate an eosinophil from a polymorphonuclear leucocyte. However, I think if the method introduced here by Dr Beacom is followed out and checked by this method we could get a fair idea of the accuracy. Undoubtedly when we use the dilution of the blood for the total counts we get a more even distribution of the cells, and therefore the differential counting is more accurate except for differentiating eosinophiles and basophiles from neutrophiles which in most cases is unimportant.

Dr Nathan Rosenthal—I should like to ask if Dr Beacom noticed any difference between blood smears taken from the finger and from the ear. In certain cases of subacute bacterial endocarditis microplagues may be found in the ear blood and are seldom present in blood smears taken from the finger. We have found that cover slips are not suitable for the study of abnormal blood cells and have obtained better results from the use of slides.

Dr Beacom (closing)—I am very glad that Dr Sanford has tried out this method. I might state that I brought a few slides and cover slips with me and I will be glad to show

this technique sometime during the course of the meeting I did not do any work on blood from the ear. This would involve too much work to bring out the points which I wished brought out. As to the necessity of counting three hundred cells, I don't believe I have anything to say about that, since a count of 100 cells is not enough for accuracy. It seems to me that since we are doing clinical pathology, we should be as accurate as possible. It is true that a count of 55 per cent or 65 per cent polymorphonuclear means nothing whatsoever, but if we have an abnormal case of fifteen or twenty thousand and then get a polymorphonuclear count of 70 per cent or 85 per cent or more, it does mean something. Although we may have a great many slides to count, it is necessary to be as accurate as possible. The slide and cover glass method I do not give as a remedy for all evils of differential counting. Three hundred cells should be counted. There are too few clinical pathologists, and as a result most of our medical students are going to be located in towns away from laboratories. They are going to have to do their own differential counting. The cover slip method is very difficult to learn, and a medical student will not learn it. I believe a simpler method is worth while.

PATHOLOGIC LABORATORY EXAMINATIONS FOR THE DENTIST*

By CHARLES G. DARLINGTON, M.D. NEW YORK CITY

WHILE the title of this paper is given as Pathologic Examinations for the Dentist, it would have been better but too lengthy to have titled it "A Plea for Instruction of the Dentist in the Value of Pathologic Examinations."

With that object in mind, it is not my intention to burden you with a list of clinical laboratory procedures with their indications in dentistry, but rather to call to your attention an imperative duty that rests with us to teach the dentist correctly what he may expect from the use of many of our laboratory procedures.

At first thought the teaching of pathology to the dentist seems an unusually large undertaking, but not a hopeless one, in fact a rather encouraging one, but first let us consider the size of our problem.

Some comprehension of the size of the problem can be gathered from a realization of the fact that the average dentist today knows little pathology and least of all does he know of the various pathologic examinations that might be of inestimable value to him. Before we are too hasty to condemn him for his shortcomings along this line, however, we must realize that we can only condemn him according to his opportunities, therefore, on that account he deserves sympathy rather than censure as he has had little instruction in pathology, at least as compared with his medical confrere.

Now realizing that our undertaking is large, let us see how the situation is not hopeless but rather encouraging.

First, I find it encouraging, because in my experience in teaching pathology to graduate and nongraduate students the past two years at New York University College of Dentistry, I have found particularly the graduate student most anxious for knowledge along these lines. Although we must admit

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists in Washington, D. C. May 13, 14 and 16, 1927.

that while unfortunately we have not found all dental students eager for pathologic knowledge, each year we can see distinct improvement

Next, even more encouraging is the recent trend of dental education. Not many years ago the dental student was taught little or no pathology as almost all his teachings were exerted toward the mechanical side of his work. However, of recent years especially, there has been a revolutionary tendency toward emphasizing that the dentist was not just a mechanic, but, as he was working on the human body and as it was recognized that many of his patients' ills might be primary or secondary in the mouth, the doctor of dental surgery must needs have a pretty thorough understanding of much of medicine.

This has directly modified the teaching of dentistry, and naturally on this account the teaching of pathology has come more and more to the fore, and the curricula of the various dental schools are taking this into consideration. While the necessity for more extensive instruction in pathology and the other fundamental medical sciences is somewhat resented by many of the older dentists because the dentists of the past have got along perfectly well without such instruction, competition is keen, and the younger men and the older progressive men are really not only eager but anxious for such instruction.

General pathology is being taught in the dental schools but I do not believe the teaching of clinical pathology or the value of various pathologic examinations is being taught proportionately, hence this paper is written.

I do not desire to make clinical pathologists of the dentists, but a proper understanding of how and when to make smears and cultures, what assistance various laboratory examinations may render in establishing diagnoses, in particular the value of blood counts, Wassermann's biopsies, bleeding and coagulation tests, urine examinations, etc. is a definite thing that we should make it our business to have taught.

As remarked previously in this article it is not my intention to burden you with a list of clinical laboratory procedures with their indications in dentistry, but in order to help explain my previous remarks I shall select and briefly comment on urine examinations, coagulation and bleeding time tests, examinations of value in syphilis and the biopsy.

Preliminary to this, however, illustrative of the necessity for such instruction, I might put under the caption of foolish questions, remarks, or requests (remarks or requests we frequently meet)

Take a blood count for syphilis meaning a Wassermann

Take a blood count for syphilis meaning demonstration of *Treponema pallidum*

Take a blood smear for syphilis meaning a Wassermann

Take a blood smear for syphilis meaning demonstration of *Treponema pallidum*

A similar request from a smear of an oral lesion

Smear for the diagnosis of anything

Culture for the diagnosis of anything

'That patient cannot have syphilis because the Wassermann is negative''

URINE EXAMINATIONS

As I do not wish to omit the value of the simplest of laboratory examinations, urine examinations, I shall mention them, but it is not my intention to discuss them at this time. Sufficient for the present, is the emphasis that a general knowledge of the interpretation of special abnormal elements, as pus, blood, bacteria, casts, albumin, and sugar, the last mentioned especially, may show the intelligent dentist much.

COAGULATION AND BLEEDING TESTS

I have purposely included the value of coagulation and bleeding time tests to the dentists because I am not quite sure just how valuable they are or better how valuable they might be.

About a year ago Dr. Leo Winter and I published an article,* in which we called attention to this procedure in oral work, and reviewed two hundred coagulation and bleeding tests, many of which were made both before and after suprarenal and novocaine injections. The method used was Goeckel's, a method somewhat similar to Duke's method, which has the decided objection as well as advantage of being a skin puncture method. We at least showed that the injection slightly shortened the coagulation time. In that series there were no postoperative hemorrhages but since that time we have had two interesting cases.

CASE 1—12/17/25 C. T., aged seventeen, had tooth extracted six days previously. Five days after extraction during the night bleeding started and continued until morning. Patient came to the clinic, and tests were performed with these results: bleeding time—three minutes, thirty seconds, coagulation time—ten minutes.

CASE 2—12/15/25 F., aged twenty-four, gave a history of extraction of teeth about two weeks previously, following which she bled for three days. Tests were performed two weeks after extraction with the following results: bleeding time—three minutes, ten seconds; coagulation time—nine minutes, forty seconds.

Following the tests, the patient bled from the wound in finger.

Theoretically at least, what we should like to foretell from the test is a tendency toward postoperative hemorrhage. Probably we can tell that a *tendency toward* postoperative hemorrhage exists, but there are so many other extraneous factors that influence postoperative hemorrhage that we know that we cannot tell which patient is going to have a postoperative hemorrhage, however, if not routinely done by dentists, it should at least be done in cases when the patient gives a history of any form of bleeding.

It is the style today in hospitals to do at least coagulation time tests on tonsil and adenoid cases, and I must admit that from hundreds of such tests, mostly performed with this method but many of them with the capillary tube method (both skin puncture tests, I'll admit) I have yet to notice in these "T and A" cases an abnormal prolongation of coagulation time where postoperative hemorrhage developed, and needless to say, several patients in

*Winter, Leo and Drillington, Charles G. Control of Bleeding in Minor Oral Surgery Operations with Records of Two Hundred Coagulation and Bleeding Tests. Jour. Dental Res. 1924-26, v1 13.

whom postoperative hemorrhage developed showed normal coagulation times, however, the latter are more easily explained

Perhaps had venipuncture methods been used our results would have been better but obviously for such a type of case, or in routine oral work venipuncture methods are not practicable

EXAMINATIONS OF VALUE IN SYPHILIS

Not only are the fundamentally acquired and congenital syphilitic lesions much misunderstood but more so are the laboratory procedures of and in their diagnoses misunderstood misunderstood as to how they are to be performed (material used etc.) and misunderstood as to interpretation. In this respect let us consider dark field illumination the Wassermann and the biopsy

Dark Field Illumination—The fact that this examination is for the presence of the causative agent of syphilis the *Treponema pallidum*, is not well enough understood and in the same way the knowledge of which lesions such as chancre mucous patches and gummas (active syphilitic lesions) are likely to harbor the organism and therefore should be subjected to such an examination, as well as the necessity for wet material and the significance of a negative examination are also not sufficiently well realized

Wassermann—The dentist's knowledge of the Wassermann test is largely confined to the blood Wassermann and needless to say his impression that a negative test means no syphilis while a positive test (including one and two plus reactions) means syphilis needs correction. Besides this their knowledge of relative agreements or disagreements of the Wassermann with primary secondary tertiary and congenital lues will stand improvement

Brevity excusing the too arbitrary nature of the statements let us summarize some general rules for interpretation

Except in treated cases only a three or a four plus Wassermann is a positive Wassermann

A positive Wassermann (leprosy and rhus excepted) means syphilis

Twenty per cent of known syphilitics give negative Wassermans

Spinal fluid Wassermann tests are often of value in "Nerve Syphilis" where the blood Wassermann tests are negative

Chancres show only a percentage of positive agreements with the Wassermann test and these are roughly proportionate to the age of the lesion

Syphilitic secondary lesions correspond almost exactly with the Wassermann test

Tertiary syphilitic lesions correspond in 80 per cent of cases with the Wassermann test

Congenital lesions show negative Wassermans very frequently and "inherited" lesions show negative Wassermans even more frequently

The biopsy in a given case may be of value in the diagnosis of syphilis alone or in association with nonsyphilitic lesions which association is not uncommon or it may be of value in a negative way. I shall speak further of the value of the biopsy

THE BIOPSY

Last but not least the biopsy needs some consideration. I use the term biopsy here not in its narrow or exact meaning, i.e., the taking of a piece of tissue for microscopic pathologic examination, but to include the microscopic examination of all tissues or tumors (which in many cases may be preferably removed in toto without preliminary biopsy).

From our fairly large experience in examining material from our oral surgery clinic (Dr. Leo Winter, Chief) which represents three hundred and forty tissue examinations, we have been impressed with the following facts. The term epulis (from the gum) as it may include such a variety of conditions (benign giant-celled sarcoma of the epulis type, fibrous epulis, fibroma, inflammatory hyperplasia, angiectasis or angioma, osteosis or osteoma, papilloma, etc.) should not be used. As usually those cases clinically diagnosed epulis are benign giant-celled sarcoma, or a fibrous epulis, or polyp, clinically and pathologically these diagnoses are in agreement, but we have occasionally seen the typical microscopic picture of benign giant-celled sarcoma where clinically the cases were otherwise, as

1 Clinically an inflammatory reaction but no apparent tumor

2 Clinically on account of the amount of involvement an extensive malignant looking picture

The opposite we have also seen, as

1 Clinically benign giant-celled epulis which microscopically was malignant

2 Clinically a fibrous epulis which was microscopically malignant

Carcinomas are not infrequent. Twenty-three such cases have been found out of three hundred and forty cases examined, or an incidence of 6.7 per cent. Twenty of these were squamous, one basal, and one from the antrum.

Of these twenty-three cases, the lip or tongue, "the most common location for carcinomas of the mouth" was affected in only one case—an involvement of the tongue. These twenty-three cases showed involvement of various other parts of the mouth as follows:

Gums	11
Floor of the mouth	5
Antrum	1
Palate	3
Cheek	2

The preponderance of twenty-two males to one female and the relatively higher age level (oldest, eighty-four years—youngest, thirty-one years, an average of fifty-six years) are in keeping with the usual figures for carcinomas of the lip and tongue.

The association with syphilis and the value of the biopsy plus the Wassermann is brought out by Case No. 7406A which clinically gave a history of syphilis and a fairly well circumscribed mass in the cheek with a four-plus Wassermann, leading many to the diagnosis of gumma, and being corrected to squamous-celled carcinoma by performing a biopsy.

Adamantinoma are not as rare as one would expect, ten such cases having been observed.

A knowledge of the clinical picture in conjunction with the microscopic examination is essential in many cases in establishing a diagnosis. This is particularly true of cysts and granulomas. The following case of my chief, Professor Fraser, at the New York University and Bellevue Hospital Medical College, which came to him a few years ago, illustrates this most bitterly. Not knowing anything about the clinical case, a pathologist, upon examination of a microscopic tissue, had made a diagnosis of a round celled lymphosarcoma, upon which diagnosis, unfortunately, a resection of the jaw was made. With the history and clinical findings, however, it was easily recognized by Dr. Fraser as a simple root lymphogranuloma.

I have selected these last three subjects for special comment only because my own experience has been chiefly with these problems. The value of the urine examinations especially for the detection of diabetes a disease which presents many important dental problems, the too frequent diagnosis of "Vincent's Angina," the value of blood chemical examinations, blood smears, bacteriologic smears and cultures, and the laboratory aids in the diagnosis of focal infections I shall not comment upon.

CONCLUSION

In conclusion let me add that, naturally, in order to teach the dentist the application of laboratory examinations those who know them best can teach them best. Does it not seem unfortunately true in this respect that concerning many oral lesions our knowledge is decidedly limited? How few routine laboratory examinations have been made with "routine" oral lesions? Therefore, does it not seem not only possible, but probable that could at least many of the more simple laboratory examinations be more universally made, much light would be thrown on many dental problems?

75 EAST FIFTY FIFTH STREET

DISCUSSION

Dr. Robert A. Keilty—I do not like to be a chronic discussor. This is a most important problem one which we have very much neglected as was brought out this morning in the case of urine. We cannot go into detail too much. We have neglected this and the dentist ought to know these things. The internists are demanding some work on it and it is up to the laboratory man to do a great deal of this work on this matter. When anything concerns pathology or bacteriology it rightly belongs to the laboratory man.

Dr. Wm. G. Ezton—I would like to ask Dr. Darlington how far he thinks the clinical pathologist should go, working with the dentist without having a physician in the case. Some dentists go on treating doubtful conditions in the mouth by using all sorts of antiseptics they diagnose general conditions sending urine to the laboratory, etc. and yet as if they were the proper persons to treat doubtful cases. I would really like to know just how far Dr. Darlington thinks the dentist should go and how far he thinks the clinical pathologist should go in working alone with the dentist.

Dr. Darlington (closing)—As for the dentist going ahead treating cases without an attending physician, there is not a tendency to do this. They are only too glad to get the advice of the clinical pathologist. We should give them this advice.

TURBIDIMETRIC METHODS FOR SUGAR IN BLOOD AND URINE¹

By ANTON R. ROSE, M.S. PH.D., NEWARK, NEW JERSEY

IN ALKALINE solutions, potassium ferrieyanide is reduced to ferriocyanide by glucose, and this latter can be quantitatively separated from the excess ferrieyanide by precipitation with zinc or silver salts in dilute ammoniacal solutions. Under optimum conditions, the fine suspension thus formed is quantitatively reproducible and very suitable for turbidimetric measurement.

The analysis is carried out as follows: (A) clarify by shaking sample with a modified Lloyd's reagent,² (B) add 3 c.c. of ferrieyanide reagent³ to 1 c.c. of the filtrate, (C) digest for fifteen minutes in boiling water,⁴ (D) dilute with water to the 20 c.c. mark and mix, (E) to 1 c.c. of this diluted mixture add 13 c.c. of 4 per cent ammonia solution and treat with 1 c.c. molar zinc sulphate solution, (F) and measure the resulting turbidity in the Exton Junior (or wedge) Scopometer.⁵

That the zinc ferriocyanide turbidity is highly reproducible under these conditions is evident from the following: Sixteen samples of pure glucose solutions, ranging from 0.5 to 10.0 milligrams per c.c., gave an average glucose equivalent⁶ per turbidity scale unit of 330 ± 26 (average deviation 8 per cent). Nine samples of urine of known sugar content gave a similar glucose equivalent of 331 ± 19 (average deviation only 5.7 per cent). Twenty specimens of urine run simultaneously with Sumner's dimethylglycolic acid⁷ and the above methods gave an average ratio

$$\frac{\text{Turbidimetric}}{\text{Sumner}} = 1.26 \pm 0.05$$

an average deviation of 4 per cent. Comparison of thirteen samples of urine, run with the yeast, turbidimetric, and Benedict picric acid methods, showed the following average deviations from fermentation results: Sumner's, 0.7, Benedict, 0.75, turbidimetric, 0.6. While not conclusive, the comparison suggests that the new method does not register more nonglucose reducing material than the Sumner and Benedict methods. Applied to blood, the results by this method have been more like Folin's copper method than Benedict's picric acid method.

¹Read before the Sixth Annual Convention of the American Society of Clinical Pathologists in Washington, D. C., May 13, 14, and 16, 1927.

²Preliminary report from the laboratory of the Prudential Insurance Co., Newark, N. J.

³Lloyd's alkaloidal reagent is shaken in a 20 per cent solution of sodium acid sulphate filtered by suction spread thin on glass plates and dried two hours at 80° C. and reduced pressure.

⁴For urine tests the reagent consists of 330 grams of potassium ferrieyanide and 106 grams of sodium carbonate dissolved in 1000 c.c. of water. For blood this reagent is diluted five times.

⁵The digestion is preferably done in narrow tubes graduated to hold 20 c.c.

⁶The measurement of the turbidity is very simple by the use of this instrument and takes but a moment. If the cloud is too dense to read dilute with 4 per cent ammonia until a reading can be obtained and include the additional dilution in the calculation.

⁷The number by which the scale reading multiplied by dilutions is to be divided to give the glucose in the sample.

⁷Sumner, J. B. Jour. Biol. Chem. 1925, 19, 383.

DISCUSSION

Dr William G. Exton—This test has the advantage of working over both normal and pathologic ranges of sugar in urine. The colorimetric tests now available are adapted only for the normal range and when the specimen contains sugar in concentrations above the normal the tests must be repeated on diluted urines. With Rose's test if the sample is found to contain more sugar than normal it makes no difference except in calculating the scale readings and the sugar may be quantitated no matter what the amount.

Dr G. B. Kramer—I would like to ask Dr. Rose what advantage this turbidimetric method has for clinical purposes over the method estimating sugar by the polariscope.

Dr Robert A. Adduffe—I would like to say a few words concerning all of the papers thus far presented and to comment upon one angle of the situation. All of us must be impressed with the remarkable advances which have been made in the clinical laboratory along the lines of accuracy and precision. We must remember however that unless they are brought to the attention of the clinician and their clinical significance emphasized they lose much of their value. For example I have used with great satisfaction Dr. Exton's turbidimetric method of quantitating albumin in the urine, but I frequently encounter the garden variety of clinician who cannot understand so many milligrams of albumin per 100 c.c. until it is translated into traces or moderate amounts.

I don't doubt at all that there will be some of this variety of clinician at the coming American Medical Association meeting who will see Dr. Terry's new stain demonstrated and will get the impression that all that is necessary is to get a bottle of stain and a chunk of tissue and the carcinoma will pop out.

We should endeavor to emphasize consistently and continuously that laboratory tests are not tests for disease but laboratory examinations to determine the reaction of the patient to stimuli, and we should stress the fact that laboratory procedures are methods of examining the patient. And finally we must educate the clinician to interpret the reports.

Dr Wm. C. Exton—As to the polariscope I may say that it makes a great deal of difference which polariscope is used and also who uses the polariscope. The polariscopes and saccharimeters usually seen in books on clinical pathology and in clinical laboratories are almost always of an inferior and inadequate type and insensitive as compared with our chemical methods. Some of them that I have seen will hardly detect the difference between none and 1 per cent of sugar. I fear that many who use these instruments in their routine work have very illusory ideas as to the accuracy of the results they obtain especially with short tubes and when the preliminary treatment is insufficient to properly clarify and exclude other rotatory substances which occur in urine. A good polariscope with accessories costs something like a thousand dollars and demands not only skill and experience of the observer but also great care in controlling temperature, wave length and the other physical factors involved which make the technique very time consuming. Notwithstanding its perfection as an optical method I therefore regard the polariscope as being unsuited for routine urinalysis.

TRANSACTIONS

Minutes of the Sixth Annual Convention, American Society of Clinical Pathologists—Washington, D C

THE proceedings were held in the ballroom of the New Willard Hotel, Washington, D C, May 13, 14, and 16, 1927 Convention called to order by President, Wm G Exton, Newark, New Jersey

and Dr Robert A Keilty, Washington, D C, proposed changes in the constitution and by laws to be adopted at the Executive Session

Dr C E Roderick, Battle Creek, Michigan, moved that a vote of thanks be directed to Dr Wm H Moursund and the Dallas Committee for their entertainment of the Fifth Annual Convention Motion carried

There being no further business before the session, the remaining time was devoted to the reading of papers on the regular scientific program as follows

"A Study of the Micro Kahn Test in Syphilis" by Dr Robert A Kilduffe, Atlantic City, New Jersey

"The Microscopic Kahn Reaction" by Dr Francis B Johnson, Charleston, South Carolina

"Further Studies of the Kolmer and Kahn Tests" by Dr C E Roderick, Battle Creek, Michigan

Symposium of Kahn test discussed by Dr R L Kahn, Lansing, Michigan, Dr B S Kline, Cleveland, Ohio, Dr H A Heise, Umontown, Pennsylvania, Dr Robert A Kilduffe, Atlantic City, New Jersey, Dr Robert A Keilty, Washington, D C, Dr Francis B Johnson, Charleston, South Carolina, and Dr R L Kahn (closing)

"Fatalities Following the Use of Arsenamine With Report of Autopsy" by Dr Ernest Scott, and Dr R A Moore, Columbus, Ohio Read by title

"Brain Structure Changes After Treatment in General Paralysis" by Dr A M P Saunders, Dunning, Illinois Discussion by Dr G B Kramer, Youngstown, Ohio, and Dr A M P Saunders (closing)

"The Use of Injection Methods in Pathology" by Dr Ernest Scott, and Dr R A Moore, Columbus, Ohio No discussion

Friday, May 13, 1927, 2 P M

"Blood Picture of Purpura" by Dr Nathan Rosenthal, New York City Discussion by Dr Reuben Ottenberg, New York City, Dr Reed Rockwood, Baltimore Maryland, and Dr Nathan Rosenthal (closing)

"Anemia as a Factor in the Test of the Rate of Sedimentation of the Erythrocytes" by Dr Roger S Hubbard, Clifton Springs, New York (by invitation)

"Studies of Sedimentation of Erythrocytes" by Dr A H Sanford, and Dr H F Hunt, Rochester, Minnesota Both papers discussed by Dr Carl Spohr, Columbus, Ohio, Dr Asher Yaguda, Newark, New Jersey, Dr Herman Sharlit, New York City, Dr H R Brown, Rochester, New York, Dr W G Exton, Newark, New Jersey, Dr Roger S Hubbard, Clifton Springs, New York, Dr A H Sanford, Rochester, Minnesota, and Dr G B Kramer, Youngstown, Ohio

"Differential Blood Counts A Comparison of the Accuracy Obtained by Various Methods" by Dr Dean N Beacom, Denver, Colorado Discussion by Dr A H Sanford, Rochester, Minnesota, Dr Robert A Keilty, Washington, D C, Dr F B Johnson, Charleston, South Carolina, Dr Nathan Rosenthal, New York City, Dr Dean N Beacom (closing)

"Ovarian Function Its Influence on the Concentration of Calcium in Blood" by Dr Herman Sharlit, and Dr Wm G Lyle, New York City No discussion

"Purpuric Smallpox, Review of Recent Studies" by Kano Ikeda, St Paul, Minnesota No discussion

Friday May 13 1927 8 P.M.

"A Key to the Diagnosis of Neoplasms" by Dr Wm Carpenter MacCarty, Rochester, Minnesota Discussed by Dr B T Terry Rochester Minnesota

"Rapid Methods of Examining Tissue Microscopically Without a Microtome" by Dr B T Terry Rochester, Minnesota Discussed by Dr Wm Carpenter MacCarty, Rochester, Minnesota Dr Harold G Palmer Philadelphia Pennsylvania, and Dr Philip Hillkowitz, Denver, Colorado

"The Present State of Our Knowledge of Gingivitis" by Dr Robert A Kilty, Washington D C Discussed by Dr Philip Hillkowitz Denver Colorado and Dr Robert A Kilty (closing)

"The Etiologic and Specific Relationship of Foci of Infection to Certain Organic Lesions A Postmortem Study" by Dr A S Giordano South Bend, Indiana No discussion.

"The Budding of Ameba" by Dr L H Prince Washington, D C and Dr T H T Wright Palo Alto California (by invitation) No discussion

Saturday May 14 1927 9 A.M.

"Accuracy and Precision in Clinical Pathology" by Dr P V Wells Newark New Jersey (by invitation) Discussed by Dr Hermann Shurdt New York City and Dr P V Wells (closing)

"New Clinical Methods for Measuring Color and Turbidity as Applied in the Junior Scopometer" by Dr Wm G Exton Newark New Jersey No discussion

"A Turbidimetric Method for Sugar" by Dr Anton R Rose Newark New Jersey (by invitation) Discussed by Dr Wm G Exton Newark New Jersey, Dr G B Kramer Youngstown, Ohio and Dr R A Laiduffe Atlantic City New Jersey

"Signs in Normal Urine" by Dr Isadore Greenwald New York City (by invitation) Discussed by Dr A H Sanford Rochester Minnesota and Dr Wm G Exton, Newark New Jersey

"Occurrence of Lipoids in Urine and Their Diagnostic Importance" by Dr E L Miloslavich, Milwaukee Wisconsin Discussion by Dr W G Exton Newark New Jersey, Dr Mortimer Herzberg Cincinnati Ohio Dr E L Miloslavich (closing) and Dr Wm G Exton, Newark, New Jersey

"The Comparative Diagnostic Value of the Levinson Test and the Glucose Content of the Cerebrospinal Fluid in Tuberculous Meningitis" by Dr A S Giordano South Bend, Indiana

Saturday May 14 1927 2 P.M.

"Pathology of Intestinal Tuberculosis" by Alfred Blumberg Oteen North Carolina No discussion

"The Cultivation of Tubercle Bacilli" by Dr H I Corper and Dr Noy Uvea, Denver Colorado Discussed by Dr Robert A Kilty, Washington D C, Dr C E Royce Bethlehem, Pennsylvania Dr Frank W Hartman, Detroit Michigan Dr George Ives, St Louis Missouri, and Dr H J Corper (closing)

"Pathologic Laboratory Examinations for the Dentist" by Dr Chas G Darlington, New York City Discussed by Dr Robert A Kilty Washington, D C, Dr Wm G Exton Newark New Jersey and Dr Chas G Darlington (closing)

A Modification of the Technique of the Wassermann Test" by L H Cornwall, Dr D Groszberg, and Blanche C Taylor New York City Discussion by Dr H R Brown, Rochester New York, and Dr D Groszberg (closing)

Saturday May 14 1927 7 P.M.

The sixth annual banquet was held in the Willard Room of the New Willard Hotel Saturday evening, May 14, 1927, at 9 P.M. President Wm G Exton gave an address "The Relation of Clinical Pathology to Preclinical Medicine" Other addresses of the evening

were as follows "The Relation and Responsibilities of the Clinical Pathologist to the Hospital Standardization Movement" by Dr M T MacEachern, American College of Surgeons, Chicago

Remarks by Dr George K Burgess, Director of the Bureau of Standards, Washington, D C

Remarks by Dr George S McCoy, Director Hygienic Laboratory, Washington, D C

Remarks by Rear Admiral E R Stitt, Navy Department, Washington, D C

Monday, May 16, 1927, 9 A M

The business session was called to order by Dr Wm G Eaton, President The reading of the minutes of the last meeting was dispensed with, these having been published

The report of the Executive Committee was given by Dr Philip Hillkowitz, Chairman, who read the financial report and stated that the books of the Secretary were found to be correct This report was accepted by motion of the Society

The matter of the publication of an official organ was taken up and the Executive Committee reported progress Upon motion the matter was referred to the Executive Committee for consideration and to report back to the Society Discussion by Dr A H Sanford, Dr H G Palmer, Dr Mortimer Herzberg, Dr Wm G Eaton, and Dr Frank W Hartman

The next matter to be reported on by the Executive Committee was the State Laboratory question Dr Hillkowitz read a summary of a survey of the opinions of the membership on this question Discussion by Dr Thomas F Walker, Great Falls, Montana, Dr Mortimer Herzberg, Cincinnati, Dr Frederic E Sondern, New York City, Dr Wm Carpenter MacCarty, Rochester, Minnesota, Dr Robert A Kilduffe, Atlantic City, Dr H R Brown, Rochester, New York, Dr J J Moore, Chicago, Dr George Ives, St Louis, and Dr B W Rhoads, Fort Wayne, Indiana Motion was carried that the activities of the Society for the present, at least, be confined to trying to educate the medical profession to the value of laboratory methods

The report of the Committee on Exhibits was given by Dr A H Schrade, Chairman Upon motion of the Society it was accepted

Dr Frederic E Sondern, Chairman, gave the report of the Committee on Public Relations Report accepted

The report of the Committee on Research was presented by Dr H J Corper, Chairman Report was accepted by the Society

Under this report was taken up the matter of standardizing the nomenclatures for blood typing, using the Von Dungern and Hirschfeld method as suggested by Dr Reuben Ottenberg, New York City Discussed by Dr F W Hartman, Detroit, Dr F E Sondern, New York City, Dr A J Cisselman, Camden, New Jersey Upon motion the matter was referred to a Committee to be appointed by the President, with the power to act for the Society

The report of the Publication Committee was made by its Chairman, Dr John A Kolmer, Philadelphia The matter of a book of methods being published under the auspices of the Society was discussed by Dr Wm Carpenter MacCarty, Rochester, Minn, Dr A H Sanford, Rochester, Dr Wm G Eaton, Newark, Dr A G Sandblad, McKeesport, Pennsylvania, Dr H J Corper, Denver, and Dr Herman Spitz, Nashville, Tennessee Report accepted and motion carried that Dr Kolmer be continued as Chairman of the Publication Committee, second, that the committee appoint the editors and subeditors or associate editors, third, that work be begun at once simultaneously on different fields included in the scope of the book

Report of the Service Bureau Committee was made by Dr R A Kilduffe, Chairman Dr Kilduffe reported progress Accepted by Society

The report of the Committee on the Registration of Technicians was presented by Dr Kano Ikeda, a member of the Committee Report was accepted and referred to the Committee

Upon recommendation of the Board of Censors the following were elected to active membership Dr Dorn N Bereom, Denver, Colorado, Dr Lester Neuman, Washington, D C, Dr Alvin G Foord, Buffalo, New York, Dr Wm L C Spaeth, Philadelphia, Penn

Albany, Dr Everett L Bishop Atlanta, Georgia, Dr Cerner Scullard Pittsburgh Pennsylvania, Dr Oscar B Hunter, Washington, D C, Dr Regino Cook Beck Richmond Virginia, Dr Ira C Youmans, Miami Florida, Dr Gordon Priestman, Long Island, New York, Dr Manuel G Cichner, Baltimore, Maryland, Dr John W Gray, Newark New Jersey, and Dr W G Gamble Jr, Charleston, South Carolina

To associate membership Dr M N Richter New York City and Dr W N Rogers, Trenton, New Jersey

Considerable discussion took place on the subject of admitting Doctors of veterinary medicine as associate members, and a motion was carried to refer the matter to the Executive Committee

Three changes in the constitution and by laws were then read and adopted by the Society To wit

1 "A Nominating Committee of three shall be appointed by the President at the opening session of the meeting, whose duty it shall be to prepare a list of nominees for the various offices for balloting by the Secretary Additional nominations may be made from the floor "

2 "The Secretary Treasurer shall serve a term of three years "

3 Delete "Appointment of Committees under Order of Business for Executive Session "

The election of officers resulted as follows Dr A H Sanford President Rochester, Minnesota, Dr F W Hartman President Elect, Detroit Michigan, Dr H J Nichols, Vice President Quarry Heights Panama, Dr Ward Burdick Secretary Treasurer, Denver Colorado

Executive Committee, Dr Frederic E Sondern, New York City and Dr Wm G Exton Newark, New Jersey

Board of Censors Dr Reuben Ottenberg New York City and Dr C H Monlove Portland, Oregon

The meeting was adjourned

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

CANCER The Serodiagnosis of Cancer, Hartmann, H. Bull. de l'Acad. de med., April, 1926, *rev.*, 412

Technic The amount of albumin in the serum is first determined by a refractometer. If the serum is hyperalbuminous sufficient salt solution is added to bring it to a normal albuminous composition. If it is hypoalbuminous it is concentrated by heating.

The following solutions are used:

1. Solution of nitric acid (1 cc. of pure nitric acid at 36° Baumé, 100 cc. physiologic salt solution).

2. Iodo-iodide reagent (bi-sublimed iodine 1 gm., potassium iodide 2 gm., distilled water 210 gm.).

Three cc. of the nitric acid reagent are placed in a test tube and 0.5 cc. of serum is added and shaken. Five tenths cc. of the iodine reagent is then carefully added to the slight foam on the surface of the mixture. A slight precipitate forms at the junction of the two fluids. The thumb is placed over the mouth of the tube and it is shaken slightly. The precipitate gradually settles to the bottom of the tube and dissolves. Then the tube is inverted several times.

This process is repeated after the addition of another 0.5 cc. of the iodine reagent. In certain very positive cases the precipitate formed does not completely dissolve.

After a further addition of 0.3 cc. of the iodine reagent, the same procedure is followed. When all the precipitate has fallen to the bottom of the tube, the tube is inverted and is then placed right side up. When all air bubbles have ascended to the surface the contents are examined by transparency.

If the fluid is clear the reaction is negative. A slight flocculation is the threshold of a positive reaction; if cloudy, the reaction is positive.

In 145 cases of cancer there were 131 positive reactions. In 4 of the 14 negative cases the cancer had been irradiated before the serum was taken and in one other case the diagnosis of cancer of the tongue was not confirmed by biopsy.

Among 55 controls there were 47 negative reactions.

As a whole, this reaction has an equal value for the diagnosis of cancer as the Bordet Wassermann reaction for the diagnosis of syphilis.

ACETONURIA On a New Method of Estimation of Acetone and Its Application to the Problem of Normal Acetonuria, Fleury, P., and Awad, I. J. Compt. rend. Soc. de biol., March 12, 1926, *rev.*, 570

Reagent

Solution A

Mercury bichloride	13.55
Potassium iodide q.s.	36.00
Distilled water q.s. ad	500.00

Solution B

Pure soda	27.00
Distilled water q.s. ad	100.00
Take 21 cc. of A and 9 cc. of B	

1 Distillation of the urine One hundred cc (or less if it contains much acetone) of urine, acidified by 1 cc of phosphoric acid is boiled in a distilling apparatus provided with a rectifying tube and a current of steam is passed through it The distillate is received with the precautions necessary to avoid any loss by vaporization

If there is 0.50 or less acetone per liter the first 10 or 15 cc of distillate will contain all the acetone If there are greater quantities of acetone 20 to 25 cc of distillate will suffice

2 Determination of the acetone in the distillate To 5 cc or 10 cc of the distillate containing a maximum of 5 mg of acetone add 30 cc of the reagent After coagulating of the precipitate (fifteen to twenty minutes) centrifuge and decant, then the tube being placed in cold water, treat the precipitate with 1 cc of hydrochloric acid (sp gr 1.17) diluted with an equal volume of water and 5 cc of a 20 per cent solution of potassium iodide Filter through a very small filter without folds to separate the reduced mercury and receive the filtrate in a tube immersed in cold water Wash the filter three times with water using 2 cc each time To the mixture of filtrate and wash waters, add 10 cc of decinormal iodine and 10 cc of a 27 per cent solution of sodium hydrate After ten minutes acidify with 15 cc of hydrochloric acid (sp gr 1.17) diluted to $\frac{1}{10}$ its volume and titrate the free iodine with a decinormal solution of sodium hyposulphite Calculate the result Each cubic centimeter of iodine solution consumed corresponds to 0.067 mg of acetone

Results obtained with normal urine Normal urine was examined by three different methods and the results showed that with the exception of one case the acetone amounted to just about 1 mg per liter

BLOOD The Transformation of Monocytes into Fibroblasts Through the Action of Rous Virus Carrel A and Ebeling A H. Jour Exper Med April, 1926 xlii, No 4, p 461

It is well known that one of the more important cultural characteristics of monocytes is their inability to form a tissue When free to move in the medium, they always place themselves at a certain distance from one another If they are packed together by centrifugation and embedded in a film of plasma, they migrate rapidly from the coagulum When migration is impossible they die Fibroblasts, on the contrary always live as a tissue When they are in close contact they multiply actively without scattering over the coagulum They remain packed together The peripheral cells of the growing colony are generally in contact with one another or are united by their processes It is important to observe that the transformation of monocytes into fibroblasts generally occurs when on account of some change in the medium the life of the monocytes has become impossible

The monocytes transform themselves into cells capable of living under the conditions present in the culture The metamorphosis of a monocyte into a fibroblast displays the characteristic of an adaptive change which may automatically be produced by substances set free by the monocytes themselves under certain conditions

The phenomenon that occurs in cultures of monocytes inoculated with Rous virus probably has the same significance When an area of digestion occurs in the coagulum, masses of necrotic tissue appear along its edges Around these masses, the fibroblastic forms develop The monocytes that are highly susceptible to Rous virus become transformed into fibroblasts that are not sensitive to the virus and that are not even a favorable medium for the growth of the virus It seems as if there were a tendency for a susceptible cell to transform itself into an immune cell This phenomenon could be considered as an expression of the general property with which all living organisms or chemical systems are endowed that of opposing the action of a disturbing factor But the real significance of these facts can not be understood until we know whether the transformation of monocytes into fibroblasts is reversible

In normal cultures the transformation of monocytes into fibroblasts generally occurred when cells became packed together through some mechanical factors that prevented their

free migration and determined their accumulation. Various modifications of the medium, the addition of dead tissue, and of trypsin or the products of trypsin digestion, failed to bring about the transformation. The inoculation of cultures of monocytes with filtered extract of Rous sarcoma frequently determined the appearance of fibroblasts. The first change undergone by the monocytes cultivated in vitro was a large increase in their size. Later, the giant monocytes became transformed into cells that did not differ essentially from those that grow from a fragment of adult connective tissue.

BLOOD Chemical Differentiation of Races, Manoiloff, E. O. *Munchen med Wchnschr*, 1925, LVIII, 2186

To 3 cc of a 3 to 5 per cent emulsion of cells obtained from the blood clot add one drop of 1 per cent alcoholic solution of methylene blue. Mix and add 5 drops of 1 per cent alcoholic cresyl violet and again mix.

Add 3 drops of 0.5 to 1 per cent silver nitrate, stir, and add 1 drop of 40 per cent HCl. Stir well and add 3 to 5 or more drops of freshly prepared aqueous 1 per cent potassium permanganate, the amount depending upon the quality of the dyes.

Using this method Manoiloff claims that chemical differentiation of races is possible, reporting 187 correct results in 202 tests.

Cresyl violet disappears in Jewish blood which becomes blue, while the fluid stays bluish red in Russian blood. The children of mixed marriages (father Russian, mother Jewish, Polish or Armenian) gave a faster reaction than pure Russians. Intermarriage between Russians, Germans, and yellow races evidenced no difference from Russians.

The reaction is explained on the grounds of greater speed of oxidation of Jewish blood.

CANCER An Analysis of the Botelho Serum Test for Cancer, Baty, J. M., and Greene, J. A. *Arch Path and Lab Med*, August, 1926, II, No. 2, p. 217

Report of a series of tests upon 79 patients with cancer, 62 cases of various diseases, and 4 normal persons. The diagnosis of cancer was confirmed microscopically in 65.8 per cent of the cancer cases.

The test was made in accordance with directions received from Dr. Botelho directly as follows:

1. The serum is removed from the ice box, if it has been stored, and allowed to attain room temperature.

2. Three cc of the nitric acid solution is placed in a test tube 10 cm in length with a bore of about 1.5 cm.

3. To this is added 0.5 cc of the serum (corrected to 7.8 to 8 gm protein per 100 cc). This is shaken until the serum and nitric acid solution are thoroughly mixed.

4. Then 0.5 cc of the iodine solution is added. A flocculent precipitate forms at the junction of the iodine solution and the nitric acid serum mixture. The tube is shaken very gently at first, and then gradually more violently until the precipitate has been completely dissolved.

5. Then 0.5 cc of the iodine solution is added and shaken as before.

6. To this 0.3 cc of the iodine solution is added and shaken gently until the precipitate is dispersed throughout the tube. The procedure is then completed. If the contents of the tube remain perfectly clear, the reaction is negative. If there is definite cloudiness after 1.3 cc of the iodine solution have been added, the reaction is considered positive. Observations are best made in artificial light, using a 25 watt electric light bulb as a background. If the reaction is negative, the degree of negativity should be determined by the further addition of iodine solution, 0.2 cc at a time, until a permanent precipitate appears.

The authors conclude that Botelho's reaction is of no value in the diagnosis of cancer.

It depends, like many similar tests, on some change in the physical state of the blood colloids, and may give positive results on serums from patients suffering from any disease condition in which there is such a change.

LACTOSE FERMENTING BACTERIA Studies on Lactose Fermenting Bacteria Berry F and Ey L F Am Jour Pub Health, May, 1926, vol. 16, No 5, p 491

A total of 87 well waters varying in sanitary quality as determined by careful survey of the sources from good to 'poor' were examined for colon bacilli with particular reference to the value of the methyl red and Voges Proskauer reactions. A majority of these wells (198) were located in a village and therefore were probably subject to more direct contamination with human discharges because of the proximity of outdoor toilets on the same or neighboring lot. The remainder of the wells (89) were in a rural district where the danger of contamination with human discharges would probably be less but the danger from animal discharges greater than in the village wells. The percentages of methyl red positive and Voges Proskauer negative cultures in the urban and rural wells were practically the same. It is understood of course that no claim has ever been made that these two tests would distinguish between human and animal discharges, but it was thought that possibly some difference might be found in the relative numbers of fecal and soil types in the two groups of wells.

There was an appreciable difference in the percentage of so called "fecal" types in the "good" and "poor" wells in each series. This difference was apparent to about the same degree on the basis of the methyl red and the Voges Proskauer and the indol test, and it agreed rather closely with the difference in percentage of confirmed colon tests in the respective wells. It is difficult to place a strict interpretation on these figures because there is no established scale of expected frequency of the fecal and soil types as we progress from a grossly polluted water to one of excellent quality. The important point is whether too many "good" wells show fecal types as established by the differential tests. The percentage of fecal types in the "poor" wells is of minor importance for these supplies would be condemned on the sanitary survey alone.

The striking feature of the results in both rural and urban wells was the number of 'MR+ VP-' cultures in the 'good' wells and particularly in the 'very good' drilled wells. It is scarcely conceivable that 70 per cent of the colon group bacteria in these supplies are actually of fecal origin. The only satisfactory explanation which conforms with our conception of safe well waters as determined by field sanitary survey is that many of the cultures constituting this 70 per cent are soil types and are therefore of slight sanitary significance. This confirms Koser's conclusion based on his sanitary survey of the waters he examined and on comparative tests with his citrate medium.

Without attempting to attach too much importance to the indol results in this series it is perhaps worth noting that the lower percentage of indol positive cultures correlates more closely with the sanitary survey than does either the methyl red or Voges Proskauer reaction.

Conclusion—Absence of methyl red positive and Voges Proskauer negative members of the colon group in ground waters of good sanitary quality is significant but the presence of this type in such waters cannot be regarded as conclusive evidence of fecal contamination. These differential tests, therefore, have very limited value in the analysis of well waters.

TUBERCULOSIS Tubercle Bacilli in Tuberculosis Pus Gardner A. D. Lancet, June 5 1926 1090

Gardner believes that the frequent failure to find tubercle bacilli in pus is due to unsuitable counter stains and that if a yellow counter stain (half saturated aqueous picric acid) is used the organism may be found in 90 per cent of cases.

PHAGOCYTOSIS Type of Phagocytic Cell and Its Relative Proportions in Human Bone Marrow and Spleen as Identified by Supravital Technic with Special Reference to Pernicious Anemia Doan C. A. Jour Exper Med March 1926 vol. 41, No 3 p 289

In general, there is a reversal of the normal in the ratio of plasmatocytes in the spleen to plasmatocytes in the bone marrow in pernicious anemia with a marked tendency toward the phagocytosis of young immature, nucleated red blood cells in the bone marrow. The peripheral blood picture suggests that the cells had never been in circulation. The observations made do not indicate that the spleen takes any directly active part in an increased destruction of blood in pernicious anemia.

GRANULOMA INGUINALE The Etiology of Granuloma Inguinale, McIntosh, J. A.
 Jour Am Med Assn, September 25, 1926, LXXVII, 996

This study of the etiology of granuloma inguinale was undertaken for the purpose of determining the nature, transmissibility and differential diagnosis of the causative agent and the period of the infection

Fifteen spontaneous cases furnished the material for the study reported, typical Donovan bodies being found by direct smear in fourteen cases and pure cultures being grown from eight cases

Biopsy material was fixed in equal parts of 10 per cent formaldehyde and 25 per cent aqueous potassium bichromate and stained with hematoxylin and eosin. There are four camera lucida drawings of the pictures seen

The formol gel test first described by the author in this disease (Memphis Med Jour, 1925, 11, 139) was positive in fourteen cases

The technic is as follows

To 1 cc of patient's blood serum is added 1 drop of 37 per cent formaldehyde, and mixed thoroughly. Evaporation is prevented and the mixture allowed to stand for forty eight hours. If positive, there is opacity and gelling of the serum, in some cases the gelling occurs in less than ten minutes

The organism was found to grow best on Sabourand's medium

The cultivated organism was pleomorphic, coccoid to bacillary in shape, nonmotile, non sporulating, from 0.5 to 2 microns in diameter, Gram negative, and stained readily with Wright's stain. Primary cultures, vitally stained with brilliant cresyl blue, suspended in 0.85 per cent saline solution, showed zooglear matrix, embedded coccoid forms and numerous, unequal tetrad grouping. The impression was obtained, from a study of the vitally stained organisms, that many of the coccoid bodies multiplied in a line assuming a bacillary form, some of which would swell into large, oval bodies 3 or 4 microns in diameter. The coccoid form embedded in gelatinous nongranular matrix was the most constant, and those just mentioned were inconstant findings. The anaerobically grown organism was generally larger than the one grown aerobically. The organism grown on Lemco medium was oval to bacillary in form and showed the most marked variation in size. Threadlike filaments were observed in cultures on this medium

Two instances of successful inoculation in the human being are reported

The study reported in this paper tends to prove that the Donovan body is the cause of granuloma inguinale by fulfilling the generally accepted criteria of specificity first stated by Koch. First, the organism was demonstrated in the lesion in fourteen out of fifteen spontaneous cases. Second, it was obtained in pure culture in eight of these fifteen cases. Third, a tissue graft from a spontaneous case in which Donovan bodies were demonstrated and from which they were isolated in pure culture reproduced the disease in an individual not previously exposed in any way to the possibility of spontaneously contracting this disease. Fourth, pure culture of the Donovan body was again obtained from this experimental lesion. And, further, antibody production against the cultivated Donovan body was demonstrated by the presence of agglutinins, precipitins, globulin changes and skin sensitiveness in the spontaneous and experimental cases

This is the first reported instance of successful experimental transmission of granuloma inguinale from one individual to another

The data support the belief that the Donovan body is the cause of granuloma inguinale and that it is a bacterium unrelated to the Friedlander group of organisms

Repeated exposure of normal individuals through coitus to those suffering with granuloma inguinale without their contracting the disease suggests that an actual break in the skin surface is necessary for successful inoculation or that the organism is infective only for susceptible individuals

The growth of the Donovan body in rather low dilutions of antimony and potassium tartrate may indicate that the action of the drug within the body may be indirect rather than direct

The blood globulins are disturbed either qualitatively or quantitatively, or both, in granuloma inguinale. The disturbance may be determined by the formol gel test

Brilliant cresyl blue dye dissolved in physiologic sodium chloride solution is a rapid and satisfactory stain for Donovan bodies when used without fixation and dehydration. Gentian violet given intravenously was found to inhibit the progress of the disease. It is much less effective however than antimony and potassium tartrate.

EPIDEMIC HICCUP Further Studies on the Etiology of Epidemic Hiccup (Singultus) and Its Relation to Encephalitis. Rosenow E C. Arch Neurol and Psychiat., June, 1926, xv, 712.

The results of previous studies have been verified in detail in two additional series of cases. A streptococcus alike in morphology and with similar cultural characters and having similar immunologic reactions was isolated from the infection atrium in twenty additional cases of epidemic hiccup, and with each strain, spasms of the diaphragm or other muscles were produced in animals. The organism was isolated from these animals and characteristic symptoms again induced on inoculation. It was demonstrated in the lesions, and proved absent elsewhere by microscopic examination of sections. Similar experiments, made with streptococci from cases of poliomyelitis and other diseases of the nervous system, from poliomyelitis contacts, from normal controls and from patients that had recovered from hiccup, gave strikingly different results. The possibility of an accompanying filterable virus (in the usual meaning of that term) being the cause of the spasms was excluded by the successful reproduction of characteristic symptoms with some of the strains after many rapidly made subcultures, with the dead streptococci and with filtrates of active cultures; the symptoms in the case of filtrates beginning in from one to three hours after injection.

Positive results were obtained by methods in which the conditions in patients were closely simulated, as well as by intracerebral inoculation of cultures, in some instances in more than one species of animal. Packing of the nose with gauze soaked in cultures sufficed to provoke spasms of the diaphragm and other muscles. Of the common laboratory animals, rabbits were found most susceptible, but similar results were sometimes obtained in guinea pigs and monkeys (*Macacus rhesus*).

The streptococcus produces in the throat of the patient, as in cultures, a filtrable substance which has the power of inciting spasms of the diaphragm and other muscle. The type of disease and the lesions induced were in many respects similar to those noted in the spontaneous disease. The spasms of the diaphragm have been seen through an incision in the abdominal wall made under local anesthesia, and by means of the fluoroscope. They were usually severe, and continued for hours and sometimes days. In the animals as in man asynchronous spasms of the abdominal muscles frequently occurred. During the severe spasms audible hiccup was frequently noted in rabbits and monkeys. The conclusion that epidemic hiccup is due to a streptococcus (*Streptococcus singultus*) having peculiar neurotropic properties seems warranted.

The close relationship believed to exist between epidemic hiccup and epidemic encephalitis and indicated by epidemiologic findings receives much support in these experiments. The symptoms and lesions in the animals that succumbed were similar to those in fatal cases of hiccup reported in the literature. In some instances, especially after one or more passages through animals, and after many subcultures, the streptococcus from cases of hiccup no longer produced spasms of the diaphragm but instead lethargic or other forms of encephalitis. Spasmodic torticollis, which developed as a late manifestation, was as common in the hiccup series as in the encephalitis experiments. The strains from the two diseases, culturally indistinguishable, are cross agglutinated and the cleared nasopharyngeal extracts in a sodium chloride solution cross precipitated by the respective antistreptococcus serums, and the encephalitis hyperimmune horse serum had marked curative effects in animals having spasms of the diaphragm following injection of the streptococcus from cases of hiccup. The results from serum and vaccine treatment of cases while striking, are far too few to be conclusive. The facts however, that the serum had a marked curative effect on animals under controlled conditions, that normal horse serum had no effect and that active immunization afforded protection for rabbits injected with the hiccup streptococcus afford a rational basis for the attempts at passive and active immunization.

DIABETES MELLITUS The van den Bergh Reaction in Diabetes Mellitus, Rabinowitch, I M Frith, A B, and Wyld, K Brit Jour Exper Med, June, 1926, vii, 155

The purpose of this communication is to record the observations which were made with reference to the van den Bergh reaction in 130 cases of diabetes mellitus. The results obtained further emphasize the relation which exists between diseases of the biliary passages and diabetes, and also demonstrate the value of this test in the management of the latter disease.

The van den Bergh reaction, known to be a sensitive test for the detection of excess bilirubinemia, was applied to a group of individuals with biliary disease.

The incidence of positive reactions found suggested that the liver escapes injury in a minority of such cases only.

Because of this finding and the known relationship between disease of the biliary passages and diabetes, the reaction was observed in a series of diabetics.

Of a group of 130 diabetics, positive van den Bergh reactions were found in 34 cases—an incidence of 26 per cent. This incidence agrees closely with that found in cholelithiasis in the 68 cases of diabetes observed by Jones, Castle, Mulholland and Bailey.

Since gall bladder disease is proved to be an important etiologic factor in the production of diabetes, and in view of the fact that as compared with diabetes, it can be more simply, and certainly more satisfactorily treated, the necessity of a simple test for its detection is obvious. The van den Bergh reaction is of value for this purpose. The routine performance of this test, may, therefore, be of assistance in the prevention of diabetes.

As with all other laboratory tests, it is only of value when properly interpreted. For the latter purpose, correlation with the clinical picture is essential in order to exclude conditions other than gall bladder disease and pancreatitis (hemolytic processes, heart failure, etc.) which may cause positive reactions.

PNEUMOCOCCUS Immunity to Pneumococcus in Rats Produced by Feeding Them the Germ, Ross, V Jour Immunol, September, 1926, vii, No 3, p 237

Rats which are fed the living pneumococci from 50 c.c. culture per day are considerably more resistant to intraperitoneal injections of the same organism than are untreated control animals.

The degree of protection is as good, although not so regular as, that previously reported as having been obtained when the tissues of animals killed by pneumococcus were fed.

Rats which are fed the heat killed pneumococci (80° C two hours) in the same quantities show a decidedly inferior degree of immunity.

In a second paper (Immunity to Pneumococcus Produced in Rats by Feeding Tissues of Animals Killed by the Same Organism, Ross, V, Jour Immunol, September, 1926, vii, No 3, p 219) it was found that rats fed on tissues of animals killed by pneumococcus Type I show an increased resistance to intraperitoneal injections of the same germ.

The protection seems to be type specific.

Protective substances exist in the sera of the immunized animals.

CEREBROSPINAL FLUID Is the Sugar Content of the Cerebrospinal Fluid Increased in Pregnancy and Myoma, Wellmuth, K Deutsch med Wchnschr, May 7, 1926, li, 735

In 45 cases of normal pregnancy, 45 normal nonpregnant women, and 25 cases of myoma, normal cerebrospinal sugar values (0.005 to 0.0075), were found with the Folin Wu technique.

In 5 cases of myoma the values were at the upper limit of normal in both blood and spinal fluid. In these cases there had been severe hemorrhage and in view of the fact that a relative increase in blood sugar has been observed after venesection, this is explainable by the hemorrhage.

The results do not confirm the observations of Vogt that cerebrospinal sugar is increased in myoma and pregnancy.

BLOOD CALCIUM The Calcium Content of the Blood in Leprosy Concepcion I and Salcedo J Jour P I Med Assn May 1926 111

Determination of the calcium content of the blood of patients in various stages and of different forms of leprosy under treatment revealed that the values for calcium in all the cases examined, except paroled and suspect cases were within normal limits. The average calcium found in cases of less than one year duration was much less than in those of more than one year duration. No marked difference was noticed in the two types of leprosy. The slight increase in the calcium content observed in cases that have recovered and have been paroled (Nos 30, 31 and 32) was not due to the effect of the injection of ethyl ester.

NEPHRITIS IN PREGNANCY A Clinical Study of Nephritis in Cases of Pregnancy Rockwood R, Mussey R D and Keith N M Surg Gyner and Obst March 1926, 342

A study of one hundred cases in which renal damage occurred during pregnancy. Only fifty-seven cases in which there was hypertension and nephritis are discussed at length, the remaining 43 cases of pyelitis or pyelonephritis being omitted.

Many of the toxemias of pregnancy are associated with nephritis and can be classified as are other types of nephritis not necessarily occurring in pregnancy. The classification of Volhard and Fahr is followed.

The course of fifty-seven cases during pregnancy is followed, together with the fate of the mother and child over a period of three years.

Both nephritis and toxemias of pregnancy seem to be general diseases affecting the cardiorenal vascular system as a whole.

When the toxemia of pregnancy is classified by the same method which Volhard uses for nephritis, a marked difference in the end results is seen and this difference allows the physician to make a more accurate prognosis both as to the mortality among the mothers and as to the fate of the child in subsequent pregnancies.

TYPHOID FEVER An Unusual Case of Typhoid Fever Ross K C Med Jour Australia, May 22 1926 : No 21 p 580

A case clinically typhoid and proved to be so at autopsy in which positive agglutination tests could not be obtained during the disease. An agglutinable typhoid bacillus was obtained at autopsy.

HEPATIC CIRRHOSIS The Glycemia Curve in Cirrhosis of the Liver Puxeddu E Clin Med Ital March April 1926 111 174

The author studied the glycemia curve throughout the day in five patients with atrophic cirrhosis of the liver and in two normal subjects as controls. In each of them he compared the glycemia curve with the glycosuria.

He found that in patients with cirrhosis of the liver and other liver diseases the glycemia during fasting is normal, oscillating around 1000. The glycemia curve shows marked oscillations in patients with cirrhosis which are entirely lacking in the controls in whom the line runs more slowly and regularly. There is also in the cirrhotic patients a hypoglycemic period preceding the ascending part of the curve. The hyperglycemic curve in cirrhotic patients generally shows a sharp rise, while the descending phase is slower and does not reach the starting point until after three hours and a half or even more. The hyperglycemia test should be used systematically in testing the glyceregulating function of the liver instead of the alimentary glycosuria test which is not reliable and is always influenced by numerous outside agents.

MALIGNANT ENDOCARDITIS Apparent Mutation of Streptococci from Acute Malignant Endocarditis, Howell, K., and Beverley, D Jour Infect Dis, July, 1926, *XXX*, No 1, 12

From the blood of a case which was clinically one of acute malignant endocarditis there were isolated on three successive occasions two bacteriologically distinct organisms, *Streptococcus hemolyticus* and *Streptococcus viridans*. The two strains were morphologically different, the morphologic differences have remained constant over a period of two years. The sugar reactions, which were also different, have remained constant for the same period.

The hemolytic streptococcus strain seven months after isolation lost its hemolyzing quality and remained constantly thereafter anhemolytic. One constant mutation, therefore, occurred.

Brief animal passage indicated that the two strains were different. It was impossible by this experimental procedure to split off a *viridans* variant from the hemolytic streptococcus or its anhemolytic form, or the reverse. Transient variants occasionally occurred.

The immunologic reactions, although variable suggested that there was only a single strain, the hemolytic streptococcus. It is possible that the parasitic growth of two closely related bacterial strains may so alter their immunologic reactions that differentiation by such reactions becomes impossible.

Protection experiments with immune sera indicated that the two strains were different.

MALIGNANT ENDOCARDITIS, TRANSFUSION IN Antibody Response After Immunotransfusion in Malignant Endocarditis, Howell, K. H., Portis, B., and Beverley, D Jour Infect Dis, July, 1926, *XXX* No 1, p 11

In a case of acute malignant endocarditis, both *Streptococcus hemolyticus* and *Streptococcus viridans* were isolated from the blood on three different occasions.

During a period of five months the patient received twelve transfusions of immune whole blood.

Before immunotransfusion was begun his blood serum contained no agglutinin for the streptococci isolated. The opsonic index was 0.57 and the complement fixation reaction with streptococcus antigen was weakly positive.

The serum of the donors at the time of transfusion had an agglutinin titer of 1:10, 240 or 1:20, 480.

The agglutinin titer of the recipient's serum increased after each transfusion, reaching after the later ones a figure higher than that of the transfused blood.

The opsonic index of the recipient's blood serum also increased after each transfusion, as a rule running parallel with the agglutinin titer, and reaching values higher than those of the transfused blood.

Complement fixing antibodies also increased but appeared later than agglutinin and opsonin and fluctuated more irregularly.

The increase in specific antibodies of the recipient's blood, both as compared with its own original content and with that of the immune donors, is interpreted as evidence of the development of active as well as passive immunity as the result of immunotransfusion.

At variable periods, usually two to three weeks, after each transfusion the agglutinin and opsonin titers of the patient's blood dropped suddenly to low figures, reaching a maximum again usually within twelve days after the next transfusion.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building,
Richmond, Va

*Biological Relations of Optically Isomeric Substances**

THIS small volume represents the last work of the great master whose sudden death occurred only shortly after he had read the galley proof of the book. To the volume is thus added a certain historical interest for one cannot read these pages without subconsciously bearing in mind the work on isomeric compounds in which this great leader in pharmacology had been interested for many years before his untimely death.

The volume consists of eighty pages and represents the third Dohme Lecture delivered at the Johns Hopkins Medical School in 1921 under the auspices of the Dohme Lectureship founded by Mrs Charles F Dohme in memory of her deceased husband.

The book is divided into five sections as follows: Introductory and Historical Relation of Enzymes and Optically Active Bodies; Decomposition of Isomers in Living Tissues; Pharmacologic Action of Optical Isomers; General Aspects of the Pharmacologic Action and Influence of Configuration on Pharmacologic Action.

To those familiar with Cushny's work on the hyoscyamine-atropine and the related tropines the dextro and levorotatory adrenalins etc. this volume will come as a fitting summary of one of the most important pieces of research contributed by the author. To Cushny's mind the varying reactions (concurring for or not in turning) between dextro and levorotatory compounds (either separately or in racemic form) and optically active elements occurring in the constitution of living tissues afforded one of the best and probably one of the most accessible keys to the solution of the general problem of drug reactions. And one can read between the lines the unexpressed wish of this true scientist that the work which he had so devotedly and hopefully pursued along these lines for many years might be carried forward vigorously and faithfully by other hands.

The book deals more with theoretical than with practical considerations. But to offset this the author, half apologetically reminds us that the purely theoretical observation of the action of the pancreas on carbohydrate metabolism by A. Mering and Minkowski led to the introduction of insulin. And the casual observation of Brunton (while watching Gungee study methemoglobin formation) that nitrites lower blood pressure led to the introduction of these compounds into practical medicine. And perhaps even farther fetched was the observation of Liebreich who discovered the hypnotic action of chloral while he was engaged in a study of the decomposition of organic substances by the tissues of the body.

Symbiontism and the Origin of Species†

THIS little volume contains 141 pages and is made up of ten chapters whose headings are as follows: Introduction; History of Mitochondrial Research; The Bacterial Nature of Mitochondria; The Behavior of Mitochondria; Symbiontism; Microsymbiosis; An Analysis of Symbiotic Reactions; Symbiontism and the Origin of Species; Symbiontism in Relation to Heredity and Development; and Symbiontism and Organic Evolution. There is also a nine page bibliography.

Biological Relations of Optically Isomeric Substances. By Arthur R. Cushny M.A. and LL.D. F.R.S. late professor of Pharmacology and Materia Medica in the University of Edinburgh (Formerly in the Universities of Michigan and London). Cloth, \$2.00 paper \$1.00. The Williams and Wilkins Co. Baltimore 1926.

†*Symbiontism and the Origin of Species.* By Evan E. Wallin Professor of Anatomy School of Medicine University of Colorado. Price \$3.00. Williams & Wilkins Co. Baltimore 1926.

NOTE: To so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

The author states that the mitochondria, tiny bodies found in the cells of all living things, are themselves living organisms. The presence of such microbial invaders has been known, in many instances, to give rise to new tissue and organs. The mitochondria, therefore, may be the clue to unraveling the perplexing mystery of the origin of species.

Two points of view are held in regard to the nature of these microscopic mitochondrial structures, and this circumstance appears to be responsible for the diversity of opinion as to the activities of these bodies. The most commonly accepted view holds that mitochondria are cell organs derived from the cytoplasm. The other view, that they are microorganisms "symbiotically" united to the cell, has attracted only a few adherents and apparently has been looked upon as a fantastic and improbable theory.

During the past seven years the author has investigated the nature of mitochondria, and has arrived at the unqualified conviction that these bodies in the cell are bacterial in nature. It has been evident that a large number of biologists have been skeptical of the results obtained in these investigations, and it also appears that many have been opposed to the fundamental conception that mitochondria are bacteria. It is the chief object of this book to summarize the evidence for the bacterial nature of mitochondria, and to present some of the evidence that demonstrates the fundamental rôle played by bacteria in the origin of species.

The book is interesting and stimulating and covers a wide range of biologic phenomena. Obviously it would be most unusual if all the hypotheses that are advanced in the book should in the end prove to be correct. But nevertheless they may serve as a splendid intellectual ferment to stimulate biologists, pathologists, and bacteriologists to further and more comprehensive researches. Incidentally the book serves to emphasize in a very striking manner the great divergence of present day "anatomic research" from that which was carried on in the average medical school of two or three generations ago.

*A History of Medicine**

H. G. WELLS, in the introduction to *The Outline of History*, comments upon the unsatisfactory condition of the teaching of history as a part of a general education due, in part, to the lack of available time for a generalized study, and in part to a narrow and partial approach to this subject. Whether Wells has been successful in his attempt to treat history as a whole is neither here nor there for present purposes. His contention in the above paragraph, however, is difficult to deny in regard to history in general and cannot be disputed with regard to medical history.

It is a matter for thought, and even an occasion of surprise in these days of scientific and even ultrascientific medical education that so little time is given to, and so little interest aroused in the history of this, one of the greatest of the professions. It is surprising that only within very recent years have Chairs of Medical History been established in a few of the larger schools, and it is to be deplored that so little, and sometimes, indeed, no place is given to medical history in the curriculum of others. To paraphrase Wells, some of this neglect is undoubtedly due to the difficulty of presenting a satisfactory survey of the subject in a limited time, for it is manifestly impossible to consider medical history as the history of Hippocrates, plus the history of Galen, plus the history of Empedocles and so on, even though one had an influence upon the other and all upon the development of the medical art in general.

For the individual deeds of these worthies special texts should be consulted, but to appreciate the compelling interest of the evolution of medicine, a broad and comprehensive survey, a panoramic and philosophic consideration, is required as a starting point for interest leading to collateral reading.

*A History of Medicine From the Time of the Pharaohs to the End of the Eighteenth Century. By C. G. Cumston M.D. Lecturer on the History of Medicine in the University of Geneva, with an introductory essay by F. G. Crookshank M.D. Cloth. Pp. 390. 24 Plates. Price \$5.00. Alfred A. Knopf New York.

Such a survey is the purpose of Dr Cumston's book which is one of a number of volumes, some written and some to be completed comprising as a whole a history of civilization, whose purpose is to present a universal survey of the history of mankind.

The necessity for such a book as Dr Cumston's and the justification, if any were demanded, for the labor time and scholarship expended upon its preparation is forcibly propounded in the introductory essay by Dr Crookshank upon *The Relation of History and Philosophy to Medicine*.

Excellent as is the history this essay is no less admirable and if nothing else in the book were read, its purchase would have been profitable.

Its opening paragraph is as follows:

"Whilst the successful man the competent practice of Medicine—an Art which includes that of Surgery—may be and often is compatible with ignorance of the History of Medicine he is the best physician in the classical and fullest sense of the word who unites a mastery of his Art to an intimate acquaintance with the great historical doctrines and the philosophies upon which they are based."

With this as his thesis Dr Crookshank develops the thought that all too often the modern practitioner is but ill equipped for intellectual expansion and in a sense anticipates what Cumston says in his introductory chapter that "the abuse of erudition certainly need not be feared in medicine at the present time."

Dr Cumston's book consists of thirty-one chapters in which the history of medicine is surveyed and recounted not as a history of successive or selected individuals but as an interlocking series of complementary personages and events, each with an influence not only on his own time but on ours and on the evolution and development of medicine as a whole.

The style is clear and crisp, interesting and readable, and amply reflects the scholarly ability of the author, it continually reminds us of Littré's dictum "There is nothing in the most advanced contemporary medicine whose embryo cannot be found in the medicine of the past."

There are few who will read this book without interest and none can read it without profit. It is a book to buy not to borrow.

The plates are all of historical interest and unalike from the author's collection. There is an excellent index.

In typography and make up the book is an example of excellent craftsmanship.

The foreword quotes from Andrew Lang and with his words this comment can conclude "The little present must not be allowed wholly to elbow the great past out of view."

*The Treatment of Chronic Deafness**

WHILE this small book of eighty-five pages has reached the dignity of publication by the Oxford University Press, the contents remind one of the instruction books furnished with various electrical treatment outfits. The instructions for the use of the "electrophonoids" are extremely minute.

Dr Zund Burguet, who designed the apparatus is not a medical man but a doctor of science and the apparatus itself was devised primarily to educate the deaf mute.

Dr Cathcart became interested in it through its effect on his own disability and recounts the history of one hundred cases including nerve deafness, chronic otitis media, otosclerosis, etc., in all ages, 69 per cent of whom were definitely improved after treatment."

The author forestalls his critics who will say that the method is unscientific by reminding them that all science is founded on empiricism and that certainly any treatment that holds out hope in chronic deafness deserves a trial.

The author states that the mitochondria, tiny bodies found in the cells of all living things, are themselves living organisms. The presence of such microbial invaders has been known, in many instances, to give rise to new tissue and organs. The mitochondria, therefore, may be the clue to unraveling the perplexing mystery of the origin of species.

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the tide has again changed and carbon monoxide with much accompanying stench has now polluted the atmosphere of the uttermost parts of the earth. Indeed in many of those spots where the sky is still clear and the air is pure the future holds some promise that by and by man's flying propensities in the form of fast mail delivery or of cigarette advertisements, or perhaps in the admonition to heed the virtues of certain political aspirants or the latest forms of hair restorers may still further extend the influence of this deadly gas.

Chemically the cases have been divided into acute, chronic and relapsing. And among the latter are to be found a variety of delayed neurologic phenomena often especially characterized by central or peripheral paralyses, speech disturbances, loss of memory, etc. The peculiar mental disturbances, the involvement of the lenticular nuclei, etc. in a way remind one of the old time cases of severe chronic lead poisoning.

Since the publication of Claude Bernard's work it has almost universally been believed that carbon monoxide acts in the body solely by forming a firm combination with the hemoglobin of the red corpuscles. This results in preventing the corpuscles from carrying oxygen to the tissues and consequently the acute symptoms are practically identical with those of a slowly induced asphyxia with the exception of the color of the blood which is bright red as the result of carbon monoxide hemoglobin formation. This view has not passed entirely unchallenged however for Vahlen, Huebner, Geppert, Giacosa and others have from time to time pointed out certain features of the action of carbon monoxide either when pure or when mixed with other substances as in illuminating gas, etc. which do not apparently exactly coincide with results which might be expected to be produced by asphyxia pure and simple.

Haldane¹ (J. S.) believed long ago that he had demonstrated completely that a mouse which was placed in pure oxygen under two atmospheres pressure and whose blood would therefore absorb sufficient oxygen to render the animal independent of the specific respiratory action of the hemoglobin could be treated with any amount of carbon monoxide without being poisoned thereby.

Recently, however, new ground has been broken in this field. Warburg has found that when the proportion of CO to O₂ in a gas mixture was raised to about 5 the O₂ consumption of yeasts and a coccus was diminished. He also showed that strong light neutralized the effect of CO. He concluded that CO combined with a catalyst in the cells with which O₂ must combine before it can oxidize other substances and that this catalyst was probably an iron compound analogous to hemoglobin. As the amount of CO needed to cause a given drop in the O₂ consumption was roughly proportional to the partial pressure of the O₂, the analogy with hemoglobin could be carried still further. And in line with this suggestion Haldane² (J. B. S.) has extended this work and added new evidence to that already accumulated. Haldane has worked with wax moths (*Galleria mellonella*) with the seeds of cress (*Lepidium sativum*), and with rats thus extending the observations to a mammalian form and thereby invoking the action of CO in the presence of hemoglobin.

Haldane subjected these animals to varying percentages and pressures of CO and O₂. The actions of the moths under very low but progressively increasing percentages of oxygen were compared with their movements with addition of CO under similar conditions. The germination (splitting) of the seeds were similarly studied. The rats were placed in an air-tight chamber and subjected to pressures of one or more atmospheres of either pure oxygen or of oxygen and carbon monoxide together. When a rat was placed in three atmospheres of oxygen and one of carbon monoxide, its behavior was almost normal, but in some cases the movements of the hind limbs were rather clumsy. In two atmospheres of O₂ and one or one-half an atmosphere of CO, movement was difficult, but there was little increase of breathing and no convulsions. It was shown by J. S. Haldane⁴ (1912) that at three atmospheres of O₂ and one of CO the rat's hemoglobin was about 98.3 per cent saturated with CO, the remaining 1.7 per cent being a very inefficient carrier of O₂. Since a single atmosphere of CO is sufficient to nullify practically all the hemoglobin, the effects of any further addition of that gas can only be on the tissues, since the addition of a physiologically inert gas, such as N₂, is without effect. The rats used in these latest experiments (J. B. S. Haldane) showed very different susceptibilities to high pressures of CO, all from the same cylinder. Thus while Rat H died on exposure to 3 atmospheres of O₂ and 2 of CO, and Rat F in 2 of O₂ and 2 of CO, Rat B appeared practically normal after four minutes in this mixture, though it died on raising the CO pressure to 3 atmospheres. And Rat D appeared little affected by four minutes exposure to 2 atmospheres of O₂ and 3 of CO, though it was killed by an additional atmosphere of CO. Haldane suggests that these differences may be due to different relative affinities of the catalyst for CO and O₂, but that it seems equally plausible to trace them to differences in the cerebral vasoconstriction caused by O₂ (Tinel, 1927).⁵ No definite evidence was obtained that poisoning by CO at high pressure can be relieved by high pressures of O₂, but attempts to prove this were rendered difficult by the fact that convulsions due to acute O₂ poisoning set in at about 7 atmospheres pressure of O₂. It was, however, clear that CO has a poisonous action on rats apart from its combination with hemoglobin. It is very unlikely that the catalyst, or substance sensitive to CO, is a mere carrier of molecular oxygen like hemoglobin. The nature of the catalyst is, of course, unknown, though it may well be an iron-porphyrin derivative. Oxygen activated by the catalyst in question may be required for the oxidation of a variety of substances. As such catalysts have now been found in bacteria, yeasts, higher plants, insects and mammals, they are presumably present in the majority, if not all, aerobic organisms.

Haldane also brings out one other point of interest and that is that all his samples of CO had a definite smell, generally compared to that of garlic or tar, and this was not changed by thorough purification. It is therefore likely that odor is a physiologic property of CO. It is only observed in fairly high concentrations, which may of course, be smelled with safety provided the operation is not repeated too often at short intervals. But the chief conclusion to be drawn from Haldane's work is that rats living on

oxygen dissolved in their blood in the presence of sufficient CO to combine with almost all their hemoglobin are killed by the addition of more CO, which must affect some substance (a catalyst) in their tissues

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—D E J

Oskar Minkowski

THE thirteenth of January 1926 will mark the seventieth anniversary of the birth of Oskar Minkowski, and we desire to take the opportunity to place on record our high regard for the contributions of this great master in medical research. We beg to offer him our congratulations and our good wishes for a happy retirement.

Minkowski ranks as one of the leaders in the investigation of the problems of metabolism by experimental methods and his paper on pancreatic diabetes published in 1893 (*Arch f Exper Path u Pharmacol*, 1893 cxvii 167) is justly considered by those who are familiar with it to be an example of what such papers should be.

Born in Koino in West Russia Minkowski moved to Kongsberg when he was thirteen years of age. Here he studied medicine and after receiving the M.D. degree, he became attached to the clinic at Naunyn who was then at Kongsberg. Shortly afterward Naunyn moved to Strasbourg and Minkowski accompanied him, being greatly interested in the chemical and experimental investigation of disease. Naunyn established in Strasbourg a laboratory of experimental medicine in connection with his clinic, thus being the forerunner of the numerous laboratories of this type now to be found both in Europe and in this country. Realizing the importance of a thorough knowledge of organic chemistry for research in this field Minkowski also worked in the pharmacologic laboratory of Schmiedeberg. About this time the idea of acidosis was forming itself in the minds of men mainly through the work of Walther and Stadelman and Minkowski discovered that in diabetes such a condition was caused by the presence of oxybutyric acid in the urine. Minkowski was greatly interested in these discoveries and it was about this time, 1889 that von Mehring who was professor of State medicine in the University, a former disciple of Ferriehs and the discoverer of phlorizin diabetes, consulted him with regard to the possibility that a fat which he called hpanin might be absorbed from the intestine without being digested. To test this possibility Minkowski proposed feeding the fat to animals (dogs) from which the pancreas had been removed. In an animal in which this was done, it was noticed that the dog was passing urine with great frequency. This suggested the possibility that diabetes might be present which was soon confirmed by obtaining a positive test for sugar in some of the urine which

had been voided on the floor. The significance of this discovery was immediately grasped by the two investigators, and for the next few years Minkowski devoted himself to a thorough investigation of pancreatic diabetes, finally publishing his results in the paper above referred to.

Since the publication of that paper Minkowski has at various times contributed further facts with regard to experimental diabetes, and indeed has so completely covered the field that little has been left for others to add. Among other things he established the significance of the D/N ratio and of the almost complete recovery in the excreta of ingested sugar. He also showed that the diabetes was not due to the absence of the digestive secretion of the pancreas, since it did not occur when a graft was left in the abdominal wall after removal of the main gland, and he suggested that the absence of some secretion normally produced by the pancreas might be the responsible cause. Failing at first, however, to accept the hypothesis of Laguesse that this internal secretion was produced in the islets of Langerhans. He tried the effect of administering extracts of pancreas to diabetic dogs, and although this part of his work was not crowned with success, his discovery of pancreatic diabetes must be regarded as an absolutely essential step leading to the demonstration and isolation of insulin.

Besides working in the field of diabetes Minkowski has contributed many other important facts in animal metabolism, for example, the origin of uric acid. It was in connection with this work that he discovered that the liver could be removed from geese without interfering with the circulation, on account of the natural anastomosis between the portal vein and the vena cava which exists in these animals. After removal of the liver he found that uric acid became replaced in the urine by urea and ammonium lactate.

It is of interest to note that a brother of Minkowski, Heimann, was one of the foremost mathematicians of his day, being one of the teachers of Einstein and contributing just prior to his premature death, a most important contribution to Einstein's theory.

—J J R M

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PATHOLOGY OF INTESTINAL TUBERCULOSIS*†

By ALFRED BLUMBERG, M.D. PH.D. PHARM.D. OTEEN NORTH CAROLINA

INTESTINAL tuberculosis may be a primary condition in children. It occasionally happens that intestinal manifestations make themselves known in adults before the pulmonary tuberculous infection is recognized. Whether such manifestations represent primary intestinal tuberculosis or are merely the result of tuberculous influences is still a matter to be proved. As a rule intestinal tuberculosis in the adult is a secondary manifestation of far advanced active tuberculous infection of the pulmonary tissue. The condition is of frequent occurrence. In far advanced active cases it may be present in from 70 to 80 per cent. In moderately advanced active cases it may be present in from 14 to 16 per cent while in the early cases it may exist in from 5 to 8 per cent.

It is assumed that intestinal tuberculosis is the result of the successful deposit and multiplication of the tubercle bacillus within the intestinal wall. The organism is carried to this place from an existing focus either by means of the lymphatics or the blood vessels or perhaps, most often through the alimentary canal by means of swallowed sputum. In children food containing tubercle bacilli may also be responsible. Why is it that intestinal tuberculosis is more frequent with the far advanced active patient than it is with the far advanced inactive patient or in moderately advanced active cases? Tendeloo¹ is of the opinion that the number of bacilli introduced into the alimentary canal is a factor in the possible creation of intestinal lesions. He believes that tuberculous tissue changes appear only when the "poisonous potency" of the organisms has reached a given optimum, as a result of which the tissue becomes susceptible and such changes result. This assumption

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists in Washington, D. C. May 13, 14 and 16, 1928.

†Permission for the publication of this article was obtained from the Director of the U. S. Veterans Bureau, Washington, D. C.

does not fully explain the question raised. If we consider that a sputum containing but two or three organisms per microscopic field will deliver many thousands of tubercle bacilli per c c, we realize that many millions of organisms will pass over the intestinal mucosa during the twenty-four hours of the day. We also realize that introduction into the alimentary canal of such organisms will not be limited to a single day, but to the passing of such sputum over the mucosa for many months without the existence of intestinal lesions. Tendeloo defines "poisonous potency" as Virulence multiplied by number of bacilli. This formula, however, is insufficient to explain successfully the cause of tuberculous extension in other parts of the body from the primary focus.

As has been mentioned, a constant supply of material rich in tubercle bacilli fails for a time to produce lesions in cases of moderately advanced active tuberculosis. It is difficult to believe that such patients fail to swallow some of their sputum during the course of the disease. Still many of these patients offer not the slightest evidence of intestinal infection. In fact, upon postmortem examination a certain number of far advanced cases show no gross tuberculous lesions in the intestines. Whether the intestinal wall in these cases is free from tubercle bacilli cannot be stated, for it may be that in spite of the absence of gross lesions some bacilli may have passed the mucosa, and that the failure to find such organisms upon histologic examination may be due to the scarcity of the bacilli and to the probability that a part was examined in which no organisms had been deposited.

It is not denied that ingestion of tubercle bacilli gives rise to intestinal tuberculosis, in fact, a wide literature tends to confirm this (for literature see Baumgarten, Bolinger, Cornett, McFadden, Kovacs, Rabinovich)¹. The conclusions drawn by the various writers are that the susceptibility is greater in younger animals, that in a number of cases tuberculous infection macroscopically confined itself merely to the mesenteric glands, that it was possible to find tubercle bacilli in the blood, lungs, liver, and mesenteric glands of the animal from seven to twenty-two hours after their having been fed with the organisms, and that the lymph follicles of the intestines are very prone to be damaged by the tubercle bacilli.

Tendeloo thinks that certain factors are responsible for the favorable deposit of the organisms in the intestines. These factors are as follows. An empty stomach or empty intestine will allow the passing through the mucosa of the bacillus with greater readiness than an alimentary canal which is not empty. Certain types of vehicles may serve the tubercle bacillus, as for instance milk, which carries the organisms and allows their deposition upon the intestines with great readiness.

I do not believe that the causes mentioned explain successfully why infection occurs with some patients and not with others. From our clinical observations and autopsy material examined, we learned that most of the patients have had some functional disturbances. It may be assumed that changes of such type predispose to successful tuberculous invasion which would not occur in a perfectly normal structure. Patients who have a chronic gastritis, who show atrophy in some parts of the mucosa of the small

intestine, or patients with extensive or localized stasis in the small intestine and such who have complained of meteorism as a result of the stasis are by far more likely to be the victims of this condition. The circulatory disturbance which gives rise to stasis results ultimately in meteorism, which again leads to increased stasis. This vicious circle prepares the intestine for the reception and the development of the organism. These changes are perhaps the result of tuberculotoxic influences which gradually alter the normal function of the cells and are responsible for the complaints of the patient. There are strains of tubercle bacilli which are able to produce tuberculotoxic products in greater quantity than do others. The individual cells of the tissues possess biochemic substances, most likely belonging to a class of fatty alcohols, which serve to protect against toxic influences. In time, however, the cells gradually become deprived of such protection. The sugar content of the cell is also reduced and an opportunity is given to the organism to invade this exhausted portion of the tissues.

I believe that the degree of virulence of the organism lies in the extent of its ability to exhaust biochemic materials from the cell, without which material, the cellular metabolism is impaired, the function to neutralize toxic substances is weakened, and the reproducing ability of the cell is diminished. The optimum expression of such virulence allows successful invasion. Many organisms possessing but a small amount of such virulence will give rise to little or no activity unless they are numerically increased to that extent where the necessary quantity of toxic substance has accumulated to inactivate cellular function. On the other hand, a comparatively few organisms may possess the ability of producing extensive tuberculotoxic substances.

Certain types of cells have a great ability to maintain their biochemic contents, others are less able to withstand outside attacks. It is quite well known that one of the easiest damaged tissues is the epithelium, which lines mucous surfaces. That such epithelium is damaged in the intestines before successful invasion occurs is assumed. Of course no epithelial destruction is necessary when the bacilli are distributed by either lymph or blood stream. That the blood stream is, at least in certain cases, responsible for the occurrence of lesions in the intestines has been shown where general hematogenous dissemination gave rise to the presence of miliary tubercles in the intestines. Such miliary dissemination in the intestines has occurred in 43 per cent of our cases. That the blood stream does carry organisms has been shown repeatedly and the probability is that organisms carried by the blood stream at one time or another will be deposited and lead to consequent tissue changes.

The lymph stream also carries organisms. The specimen is an example of a condition which is met with fairly often. It occurred in 8 per cent of our cases. The specimen shows tubercles which according to MacCallum are actually formed in the lymph channels in a manner to block the lumen. The lymphatics become conspicuous and as a result of distention by clear fluid or opaque chyle, the beaded arrangement is formed, the obstruction occurring at intervals. It is assumed that these lesions represent the path over which the organisms traveled before they reached the mesenteric lymph nod

ule which is the seat of the secondary tuberculous deposit. Whether the same appearance of the lymphatics is ever the result of the extension by retrograde process from the lymph nodule to the intestine is not known, although opinions exist that the upstream course of the tubercle bacilli is a possibility. Kaufmann² states that "tuberculous infection of the intestine may be the result of extension in a retrograde manner from the mesenteric glands to the intestinal wall. E. Edens describes and the author (Kaufmann) had also seen it."

Once successfully deposited in the intestinal wall, the organism after multiplication will soon, as a result of its activity, show gross evidence of its existence. Perhaps the earliest visible manifestation is a papillary elevation over the mucosa, conspicuous more by its pallor than by its size and elevation. This lesion is from one to one and one-half mm in diameter, elevated, domed, having a broad base and with no depression in its dome at this stage. Soon the dome flattens out, and the lesion becomes circular or oval in outline, and there may be a faint suggestion of a central dimple which in time becomes definitely visible. The formerly pale lesion is now of dark or reddish-brown color, although a fine pale line may still be noticeable at the junction of its base with the mucosa. As a rule, other similar lesions have developed during the same period. The lesions have a tendency to coalesce, and the approximated walls will by and by disappear. The manner of fusion of these lesions will act to a great extent in deciding whether the resulting ulcer will take a course along the intestinal folds or across them. The most frequent ulcer met with is the one encircling the mucosa of the intestine. The longitudinal was present in 3 per cent of our cases. The irregularity in the distribution of the small lesions will, after fusion, cause to a certain extent, the lace-like outline seen in the larger ulcer, although progressive necrosis beneath the elevated margin is also responsible for the irregularity of the marginal outline. The dimple which appears upon the "anatomically, primary tuberculous intestinal ulcer" is first minute. It may be punctiform or longitudinal. Later it becomes gently crater-like, and still later it becomes rather flat bottomed with many small tubercles imbedded in the floor which is often thickened and upon which the tubercles are frequently visible, giving rise to a granular appearance. The floor may be of light greyish color, it may be interspersed by many ivory-colored, circumscribed, small tubercles, it may be covered by organized exudate diphtheritic ulcer, or it may possess a blackish discoloration of either small or considerable extent. The discolored area may be dry, crusty, or soft and fragile. It is this type of ulcer that perforates. The greyish ulcer has the tendency to heal, and although it may often happen that the ulcer is deep, the thickness and firmness of its floor may be considerable.

I am not at this time going into details of the healing process of the intestinal ulcer. It has frequently been shown that these ulcers have a tendency to heal, and it may be assumed that certain far advanced tuberculous patients with no activity possess healed intestinal tuberculous lesions. We have found such upon postmortem examination.

The mucosa surrounding the ulcer may or may not be injected and may or may not show evidence of inflammation. When inflamed it may be bright red or cyanotic. It may be edematous and of velvety thickness. It may show points of bleeding. The intestinal wall may be firm elastic, or soft and collapsed. The serous surface may be of normal color or pale grey, grey, slaty grey or almost black. It may be mottled by the traversing vessels in which stasis exists. The organ may be glistening but usually is lusterless. The location of the ulcer if it be of some size, is recognizable upon external inspection of the intestine. There may be gentle bulging or thickening or retraction. There is usually localized peritonitis over it. The serous surface has a sandpapered appearance. The degree of stasis around the ulcer varies.

The recognition of small lesions is usually impossible upon inspection of the wall of the intestine, unless this wall is very thin and distended. We were never able to recognize small lesions from the outside. It is important to bear this in mind. Surgical removal of ulcerated lesions may promise a certain amount of benefit to the patient especially when threatened with a stenosis, but the removal of such visible ulcers does not insure the removal of other ulcers near by too small to be seen. We have seen at necropsy ulcers in the cecum and small intestine which were visible upon external inspection of the intestines. They were of the kind that even the best surgical judgment and the most conservative opinion would favorably consider operative interference with the hope of their complete eradication. Upon inspecting the open intestine however it was found that numerous small lesions existed as far up as the jejunum.

Microscopic examination of the early lesion shows the loss or partial loss of epithelial cells of the mucosa. There may be debris still covering the subepithelial tissue. The lymph follicles show evidence of infection the tubercles are found in the submucosa and chiefly composed of epithelioid cells. Giant cells are occasionally found in the more advanced lesions, but most frequently are not present during the early stage. In the more advanced tuberculous ulcer, necrosis, sometimes caseation aggregation of round celled infiltration or typical tubercles might be found. The round celled infiltration is usually in the submucosa while the tubercles may be either in the submucosa, mucosa or muscular layers. Round celled infiltration is often marked beneath the epithelium. It is also found in the serosa while tubercles might be found sometimes in the subserosa. When stained for tubercle bacilli, the organisms will be found not merely in the typical tubercle but also in the cluster of aggregated round cells. These clusters of round cells are frequently placed alongside a focus of early necrosis. The blood vessels are frequently engorged and dilated. Round celled infiltration may or may not be generalized. The loss of tissue in the mucosa submucosa and muscularis depends upon the depth of the ulcer. Shallow ulcers will show no loss of the muscle tissue although sometimes the intestinal wall is atrophied to the extent that even shallow ulcers will pass the muscle layers and reach the subserosa. At other times the floor of the ulcer will be so studded by individual tubercles that it will give rise to considerable thickening of that floor. In the healing ulcer fibrosis is a prominent feature.

Polypoid prolongation of the mucosa of the cecum and also in the colon is seen fairly often. We have seen it in 8 per cent of our cases upon necropsy. Sometimes only a few pale looking prolongations may be seen. At other times, however, the prolongations become diffused, especially in the cecum where their dense arrangement and considerable length cause almost complete obliteration of the cecal lumen in some cases. The mucosa has a shaggy appearance. The intestinal wall is thickened, firm, and rigid. A type of hyperplasia is seen chiefly in the colon, but also in the cecum. In this, the mucosa is of more or less velvety type. The organ is thickened throughout. The mucous membrane is granular and dense, yet soft and recoiling. The intestinal wall is comparatively inelastic. It is this kind of colon which is often markedly clean and free from fecal material, although the rectal ampulla as well as the small intestine is filled with stool, which at times is quite bloody. Because of the lessened absorbing surface and the probable hypermotility, the stool is not properly formed. This type of intestine will show microscopically many tubercles in the various structures. Ulcerative colitis, as a result of tuberculous infection, is also found, and tuberculous ulceration of the rectum is not unusual. A very frequent lesion is the tuberculous fistula in ano.

Colitis or ulcerative colitis, due to other causes than tuberculous infection may be present in the colon of the patient having pulmonary tuberculosis.

Perforation is a comparatively rare occurrence, considering the frequency with which ulceration of the intestines takes place. We had only three cases.

I have briefly given the pathologic picture of intestinal tuberculosis, with the intent to discuss a few points that may be of interest to the clinical pathologist who is called upon at times to assist in confirming the diagnosis of intestinal tuberculosis. The examination of a stool is usually requested in order to try to detect tubercle bacilli in it. Should such bacillus be found in a stool, its detection does not necessarily mean the existence of intestinal tuberculosis. It is a fact that the swallowing of the sputum containing tubercle bacilli is responsible much oftener than intestinal tuberculosis for the finding of the organisms in the stool. My purpose in describing the lesion both grossly and microscopically was to show that the debris which originates in a tuberculous ulcer of the intestine is of comparatively small amount, and this detritus contains either a very few tubercle bacilli or none at all. The examination of stools for tubercle bacilli, therefore, is not of great importance when the patient's sputum contains tubercle bacilli. The stool examination may offer some information, and it is of advantage to the clinical pathologist to be acquainted with the types of stool passed by the tuberculous patient.

Nothnagel³ called attention to the fact that tuberculous lesions in the small intestine may be responsible for constipation, and lesions in the colon for the diarrhea sometimes complained of by the patient. This is also the opinion of Archibald.⁴ In my experience, which coincides with A. Schmidt⁵ and Tendeloo,¹ diarrhea of the tuberculous patient may be due to intestinal

tuberculosis, the site of the lesion being either in the small or in the large intestine. Extensive inflammatory processes of the small intestine in addition to the existing ulceration will give rise to an increased secretion, thus—diarrhea. On the other hand, extensive changes upon the surface of the colon will also result in diarrhea like stools (soft—not formed) because of the lessened absorptive ability of the colon. Again, constipation is a frequent associate of intestinal tuberculosis and the lesion may be in the small intestine, or at times in the cecum or colon. When present in the colon the lesions are comparatively limited in their extent although individual lesions may be of considerable size. Ulcerations whether present in small or large intestines, where not accompanied by extensive inflammation will not be responsible for the production of excessive secretion in the small intestine or greatly lessened absorption in the large intestine. On the contrary, with a lessened output of secretion in the small intestine and with slow passage of stools over a spastic colon absorption of water will be more extensive. The stools will be drier than usual. In fact, scybalous masses may be found in the cecal pouch. Such masses when examined for occult blood will generally give a negative reaction nor does the appearance of such stools suggest the presence of blood. There is however one kind of stool which does suggest the presence of blood. It is the one which is of oil emulsion like thickness and of similar viscosity and of slaty grey color. It has the appearance of a thick fluid into which black gun powder was stirred. The test for blood will be positive to a very marked degree. Such stools may be present also in nontuberculous acute enteritis (specimen Green). The history of the patient and the fact that he has tuberculosis of a far advanced degree, with considerable activity suggests the possible diagnosis of intestinal tuberculosis. On the other hand light brown or sometimes alcoholic, smooth pasty viscous stool which, although light in color in fact sometimes of golden yellow color often contains blood that is detectable by the usual methods, is considerable evidence in favor of the possible presence of intestinal tuberculous lesions. The cow dung like soft mushy, greenish dark stool which contains considerable admixture of gas is the product of fermentation rather than a sequel of intestinal tuberculosis. Fermentation is the result of either lessened hydrochloric acid in the gastric juice or lessened enterokinase in the enteric juice. The zymogen is not activated and tryptic digestion is lessened with resulting fermentation. Coprostasis, associated with fermentation, will give rise to the complaint of alternating diarrhea and constipation.

Hypochlorhydria is a fairly frequent condition, but is not evidence of intestinal tuberculosis. Blood counts, complement fixation tests or other blood tests are of but little assistance in diagnosing tuberculous ulceration of the alimentary tract. In tuberculous anal fistula it is possible to make a diagnosis. Scrapings of the fistulous tract, when stained with carbolfuchsin, will show the presence of tubercle bacilli in about 35 per cent of all cases.

SUMMARY

1 Intestinal tuberculosis is a frequent occurrence. It is usually secondary to a far advanced, active tuberculous infection of the pulmonary tissues.

2 Ulceration appears only after certain changes in the cells, which, as a result of partial or complete devitalization, allow successful deposit and multiplication of the tubercle bacillus.

3 Early lesions may not be responsible for clinical manifestations, as complained of by the patient. In fact, such complaints may not be associated with intestinal tuberculosis, or there may be no complaints whatsoever at the time the lesions make their appearance.

4 The early dyspeptic manifestations do not represent the symptoms of intestinal tuberculosis. It is during the stage of spasticity and peritoneal irritation that symptoms arise resulting from tuberculous intestinal infection.

5 The ulcers are first small and circular, later they are large and of irregular outline. They may follow the course of the mucous fold and form the encircling ulcer. They may traverse a number of folds and then represent the longitudinal ulcer.

6 Perforation is not frequent as there is a tendency towards thickening of the floor of the ulcer.

7 In the cecum and colon there may be either individual lesions or extensive diffuse tuberculosis, with thickening of the entire wall of these organs.

8 Bleeding occurs at some time during the course of ulceration.

9 Diarrhea, due to tuberculosis, is not always a result of lesions in the colon, neither is constipation always a result of ulceration in the small intestine.

10 The finding of tubercle bacilli in the stool rarely, if ever, confirms the existence of intestinal tuberculosis.

11 The gross character of the stool should be studied when intestinal tuberculosis is suspected. The nonfermentative pasty, alcoholic, viscous stool, especially the one containing occult blood, should be looked upon with suspicion, as it may point towards tuberculous intestinal infection. The findings must agree with the clinical findings and the patient's history of active tuberculosis.

12 There is no known clinical or laboratory test by means of which a diagnosis of intestinal tuberculosis can be made.

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BRAIN STRUCTURE AND BLOOD CHANGES AFTER TREATMENT IN GENERAL PARALYSIS*

By A. M. SAUNDERS, M.D. DUNNING ILL.

THE treatment of general paralysis by inoculation with *Plasmodium malariae*, brought before the medical world through von Jauregg, has aroused wide interest and is one of the most outstanding achievements in the treatment of disease in recent years.

Since the malaria treatment has been instituted in the Illinois state hospitals, a marked change in the appearance of the wards for paretics has taken place. Formerly a case of general paralysis meant a bedridden, foul smelling, untidy patient requiring complete care for from three to five years or until he died. With the exception of a few, all of those treated patients are able to be out of bed and can take care of their personal needs. Many of them assist with the work on the ward and some of them go out during the day to work in the industrial departments. Quite a large number can go home and return to their former occupations.

It has been said that with the malarial treatment the paretics make a "good social adjustment" but no changes in the brain pathology in the blood, and in the spinal fluid have been found. During the last year, this has been found to be incorrect. Straussler and Koskinas in their study of thirty-eight brains of patients with general paralysis who had been treated with malaria have found only a very mild degree of inflammation. It is their belief that the characteristic diffuse inflammation of a typical paretic brain changes to a more localized, gummatous type resembling cerebrospinal lues. Gerstmann finds that mental improvement in those cases corresponds with changes in the brain. Nicole and Steel have often obtained negative serologic results on blood and spinal fluids in patients treated with *Plasmodium malariae* but were unable to prove any relationship between the serologic findings and the mental and physical improvement. Some patients with good mental and physical results still had a positive Wassermann and Lange test, while others with only fair mental and physical changes had a negative Wassermann on both blood and spinal fluid. This observation has been substantiated at our Psychopathic Institute. Dr. G. B. Hassin, at our Institute, has found a regression of inflammation after treatment with the malarial organism and tryparsamide. In no case was a regeneration of the parenchyma found, but the changes in the meninges and especially in the perivascular cell infiltration, have been very striking. The marked decrease or even absence, of plasma cells, which in untreated cases crowd the lumen of the cerebral capillaries, has been most interesting. The plasma cells were replaced by lymphocytes of the small cell type.

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From the Illinois State Psychopathic Institute.

Following the observation of changes in the type of cells in the brain we reasoned that it might be interesting to see if there was a definite change in cells in the blood also and we have made regular examinations of blood smears from patients with general paralysis, before any treatment was given, three months after malarial treatment, and again one year after the treatment. We have found the relative number of small lymphocytes very variable and probably influenced by the malarial organism but we failed to find any relationship in the number of small lymphocytes to the physical and mental reaction to treatment. There also was no relationship demonstrable in the number of eosinophiles to the condition of the patient. The Arneth count, however, yielded very definite and constant results in over one hundred and twenty cases showing a shifting to the right after malarial treatment, usually reaching its maximum within twelve months. In many cases the marked shift to the left found in untreated cases of general paralysis had been corrected to such an extent as to give a nearly normal Arneth count. The following charts (Fig 1) will illustrate only a few but quite characteristic and constant changes in the Arneth count before malarial treatment and at definite intervals afterward covering a period of one year. Thus shift to the right in the Arneth index was found in over 80 per cent of a total number of one hundred and twenty cases reaching its maximum after from six to twelve months after the intravenous introduction of plasmodium malariae.

DISCUSSION

Dr G B Kramer—I merely want to ask Dr Saunders how many chills a patient must have before antimalarial treatment is instituted.

Dr Saunders (closing)—We usually wait until the patient has had twelve definite chills.

A STUDY OF THE ATMOSPHERIC POLLEN AND BOTANIC FLORA OF THE EAST SHORE OF SAN FRANCISCO BAY*

BY ALBERT H ROWE M S, M D, OAKLAND, CALIF

THIS paper reports observations and deductions made throughout the last twelve months on the atmospheric pollen and on the botanic flora of the cosmopolitan area on the eastern shore of San Francisco Bay. A study of the flora of San Francisco itself shows that it differs in only a few respects from that of the East Bay district which we shall consider.

The East Bay district is in central California and extends between $37^{\circ} 6'$ and 38° latitude, from Haywards on the south to Richmond, which is thirty miles to the north. This area is bounded on the west by San Francisco Bay and is from four to seven miles in width, with the crest of the coast range hills rising to a height of from 1000 to 2000 feet at the eastern border. Along the bay shore, salt marshes are found in Richmond, Alameda, San Leandro, and Haywards. The inhabited area is either flat or moderately rolling, while the hills to the east are cut by wooded canyons that have much grass and *Artemisia Californica* and *Vulgaris* over them, and have in places pines and eucalyptus which have been planted. In one area, there are native redwood trees. The temperature varies between 40° F and 75° F throughout the year. The prevailing winds are from the west and south and occasionally from the north. The rainfall averages about 25 inches, a few rains occurring in the fall and spring and a good many during the winter period. Winters are mild, and only rarely does snow fall on the hills. The climate of this district, especially of Alameda, Oakland, and Berkeley, is tempered in the summer by sea breezes and high fogs which roll in over San Francisco Bay and through the Golden Gate across the bay, which is a body of water varying from 3 to 7 miles in width. This mild, dry climate, with the prevailing winds off the bay, explains in part the presence of small amounts of pollen in the air, especially in the summer and fall.

The East Bay region is in the center of the Franciscan botanic area, described by Jepson¹ as extending from Monterey on the south to Mendocino County on the north. This area contains many endemic plants, as well as many alien exotic species, especially grasses, which have been brought into this region chiefly from the Mediterranean countries.

PLAN OF STUDY

A combined study of the atmospheric pollen and of the botanic flora of this large cosmopolitan area was suggested by the article of Koessler and Durham² on their survey of Chicago. During the summer and fall of 1926, a botanist, Miss Weisendanger, gave her entire attention to the problems of this

*Read before the American Association for the Study of Allergy, Washington D C
May 1927

report, and plate counts of atmospheric pollen have been continued by my assistant, Mrs D Fletcher, during the remainder of the twelve month period. Our data on the pollen content of the air at four different stations in this East Bay district constitute the first data of this type gathered over a period of an entire year.

This district has been divided for convenience of study and tabulation into seven areas, by boundaries of the separate communities of this area whose total population is about five hundred thousand people. These districts are from north to south, Richmond, Berkeley, Oakland, Piedmont, Alameda, East Oakland and San Leandro and Hayward, as indicated roughly in Chart 1.

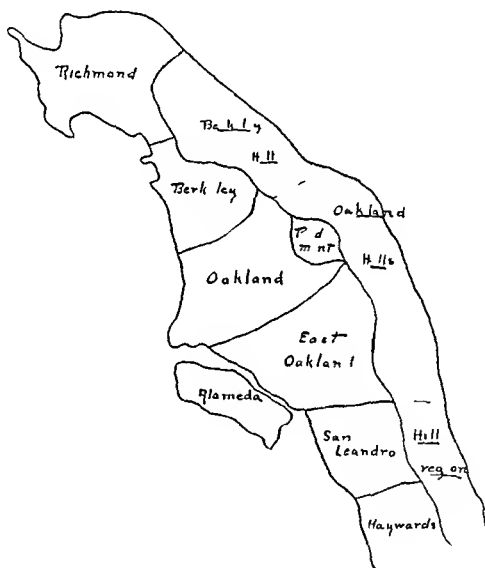


Chart 1

MORPHOLOGY OF POLLEN

In order to identify the pollens on the exposed plates, it was necessary at the outset of our work to become familiar with the size, shape, and external features of the pollen grains of all the common hay fever producing plants of this East Bay district. The articles of Wodehouse³ and Pope⁴ are the only recent contributions on the morphology of pollen. Both articles include drawings of various pollens but no definite drawings of California pollens are available. My bacteriologist, Miss Lundahl, has made careful drawings of the various types of dry pollens. These are reproduced in Chart 2 and are drawn according to scale. Pollen grains adhering to slides covered with white

vaseline maintain their dry shrunken shapes, and our studies for identification purposes have therefore been made on the dry pollens

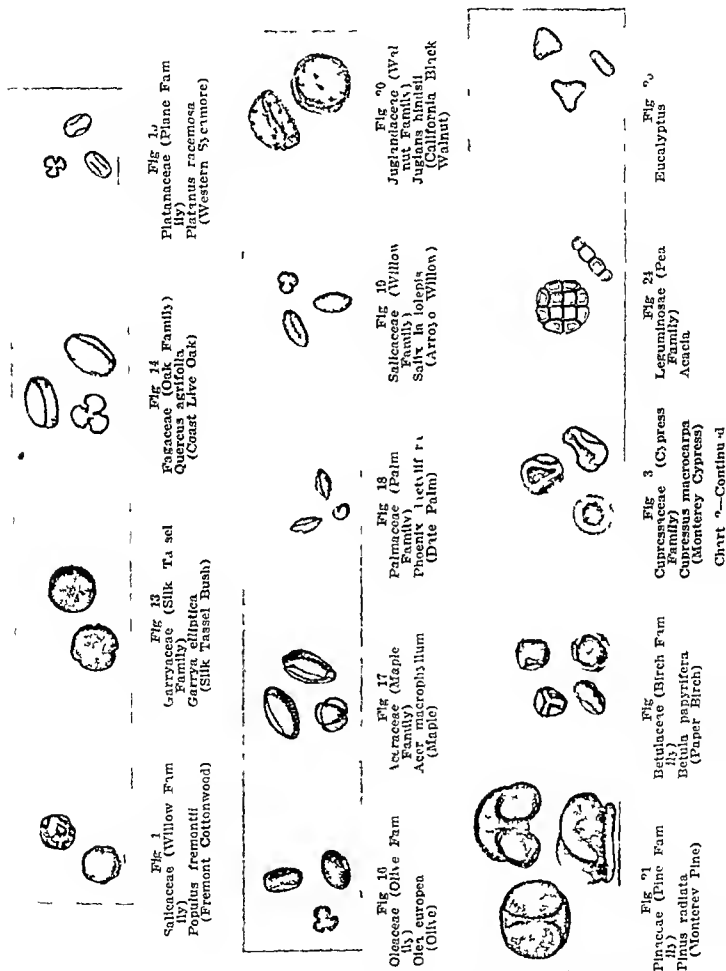
Miss Pope has classified the pollen grains of a large number of families of plants according to structural features. On the basis of her classification and on our own observations, I have classified the common hay fever-producing pollens of this East Bay District in Table I

	<p>Fig 1 Compositae (Sunflower Family) <i>Ambrosia psilostachya</i> (Western Ragweed)</p>		<p>Fig 2 Compositae (Sunflower Family) <i>Franseria biphinnatifida</i> (False Ragweed)</p>		<p>Fig 3 Compositae (Sunflower Family) False <i>Xanthium canadense</i> (Cocklebur)</p>		<p>Fig 4 Compositae (Sunflower Family) <i>Artemisia vulgaris</i> (Mugwort)</p>		<p>Fig 5 Urticaceae (Nettle Family) <i>Urtica gracilis</i> (Common Nettle)</p>		<p>Fig 6 Plantaginaceae (Plantain Family) <i>Plantago lanceolata</i> (English Plantain)</p>		<p>Fig 7 Gramineae (Grass Family) <i>Poa pratensis</i> (Kentucky Blue)</p>		<p>Fig 8 Polygonaceae (Buckwheat Family) <i>Rumex acetosella</i> (Sheep Sorrel)</p>		<p>Fig 9 Amaranthaceae (Amaranth Family) <i>Amaranthus palmeri</i> (Careless Weed)</p>		<p>Fig 10 Chenopodiaceae (Salt Bush Family) <i>Chenopodium album</i> (White Goosefoot)</p>		<p>Fig 11 Typhaceae (Cattail Family) <i>Typha augustifolia</i> (Narrow Leaved Cattail)</p>
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Chart 2

The drawings and this table of the characteristics of the various pollens indicate that it would be difficult to distinguish with certainty the identity of the spiculated pollens belonging to the Compositae family, though we feel that we can usually pick out ambrosia pollen quite accurately. *Artemisia* pollen would be difficult to separate from the ellipsoidal smooth tree pollens

were it not for the different seasons of pollination. *Plantago* and *Rumex* pollens are probably often confused, though the bands on the *Rumex* pollen and



its ellipsoidal shape are quite different from the features of *Plantago* pollen. *Amaranth* and *Chenopod* pollens are so similar that they cannot be distin-

TABLE I

TABULATION OF CHARACTERISTICS OF HAY FEVER PRODUCING DRY POLLEN GRAINS OF THE EAST BAY REGION OF CALIFORNIA

	SHAPE	TYPE	AVERAGE SIZE (μ)	FIGURE*
<i>I Shrubs, grasses and weeds</i>				
A Echinate	1 Spherical	Ambrosia	38	1
(Probably all Compositae except Artemisia)		Franseria	25	2
		Xanthium	34	3
		Iva	25	—
		Helianthus	38	—
	2 Ellipsoidal	Hemizonia	38 x 29	—
B Smooth	1 Ellipsoidal	Artemisia	38 x 25	4
	(Polyhedral)	Urticaceae	25 x 12	5
		Ricinus	46 x 33	—
	2 Spherical	Plantago	34	6
	3 Polyhedral	Gramineae	32 x 40	7
C Punctate, with Furrows		Brassica	46 x 25	—
D With Bands	1 Ellipsoidal	Rumex	46 x 34	8
E With Distinct Pores	1 Spherical	Amaranthus	29	9
F With Less Distinct Pores	1 Spherical	Atriplex	29	10
		Chenopodium	29	—
		Salicornia	29	—
G Four Grains Held Together		Typhaceae	10	11
<i>II Trees</i>				
A Punctate	1 Spherical	Populus	29	12
	2 Ellipsoidal	Garryaceae	38	13
B Three Lobed	1 Smooth			
	a Blunt ends	Fagaceae	42 x 29	14
		Plantanaceae	25 x 17	15
		Oleaceae	33 x 21	16
	(Markedly reticulate)			
	b Less blunt	Aceraceae	46 x 25	17
	c Pointed ends			
	(One Groove)	Palmaceae	25 x 13	18
	2 Punctate	Salix	29 x 18	19
		Juglandaceae	50	20
C Hemispherical		Pinaceae	63	21
D Two Winged	1 Reticulate	Betulaceae	29	22
E Polyhedral	1 Smooth	Cupressaceae	32	23
		Eucalyptus	25	24
F Triangular	1 Smooth	Acacia	42	25
G Gridiron	1 Spherical			

*This refers to figure numbers on Chart 2

gushed accurately All pollens of the Chenopodaceae family are spherical, about equal in size, and have fairly definite depressions on their external surfaces We have found Gramineae pollens of the same characteristic shape, and feel that we can identify them quite accurately on the pollen plates Our drawing is of the dry pollen and, therefore, does not show the single germinal aperture which Wodehouse points out is the distinguishing feature of the Gramineae pollens in the wet swollen condition Poa annua, Festuca myuros, and Cynodon dactylon pollens are on the average smaller than the pollen of many other species of Gramineae However, such difference in size cannot form the basis of really accurate differentiation No difficulty is experienced in recognizing most of the tree pollens, except those already mentioned

ATMOSPHERIC POLLEN

Pollen counts have been made twice every week throughout the year in four of the districts shown in Chart 1, namely, Richmond, Oakland, Piedmont, and East Oakland, and in five other locations in this East Bay region twice

every week from May to October of 1926. Microscopic slides covered with a thin coating of white vaseline were exposed in an upright position toward the prevailing wind for twenty-four hours. One square inch of each slide was carefully examined microscopically, and the various pollen grains on these areas were counted and identified. The amount of pollen on one square inch of plate surface is recorded rather than that on 0.55 square inch, as reported by Koessler and Durham, because of the small amount of pollen present in the air of this East Bay region as compared with that in the air of many areas in the middle western and eastern states.

The average counts of all stations for each day that slides were exposed throughout the year are recorded in Table II. The number of slides counted on each day is indicated, together with comment on the wind and weather. The usual occurrence of high sea fogs and the absence of many excessively hot days in this district is shown in the table. Rain occurred for a few hours on many days during which slides were exposed and used for our data. When rain continued all day, no exposed slides were counted, which accounts for a few irregularities in the routine of pollen counting in the winter.

On the basis of the studies of the morphology of the hay fever pollens of this district the pollens have been classified under the groups indicated in Table II. The reasons for such grouping of pollen grains are given in the discussion on morphology of pollen in this district.

The data in Table II consisting of 8 or 9 observations for each month have been condensed into average weekly values and are presented in a graphic manner in Chart 3. A study of this chart shows that the height of the grass season occurs in the last two weeks of May and the first week of June, due to the profuse pollination of *Lolium perenne* as shown by field observations. The chart shows that grass pollen is in the air however throughout the year, the smallest amount being present during August, September, and October. With the onset of rain in September a slight increase in grass pollen occurred. On sunny, mild days throughout November, December, January, February, and March grass pollen was found in increasing amounts in the air, and persisted through the spring. The types of grass pollen making up the average curve will be discussed under the section on the flora.

The occurrence of tree pollens in the air is tabulated separately in Table II and shown as a group in Chart 3. Such pollen begins to appear in the last week of January and continues on through February, March, April, May, and June. Pine pollen, as shown in Table II, has been found in small amounts throughout the year, due to the many species of pine found in the parks and gardens and on the hills of this district. Pine pollen apparently does not cause hay fever or asthma and consequently has not been included in Chart 3. An independent curve for willow pollen is shown during March. This pollen probably causes little if any allergy. *Rumex* and *Plantago* pollens were in the air during February, March, April, May, and June, especially in April and May, corresponding with field observations. *Chenopod* and *Amaranth* pollens were present in very small amounts during May, June, July, August, September, and October, showing that in this region these species do not produce much pollen in spite of their frequent occurrence in some districts. *Artemisia*

TABLE II
AVERAGE POLLEN PLATE COUNTS OF ALL STATIONS

	GRAMINEAE	RUMEX AND PLANTAGO	ARTEMISIA	OTHER COM POSITAE	ZEA MAYS	AMARANTH AND CHENOPOD	SALIX (WILLOW)	ACACIA	PINACEAE	TYPHA	EUCALYPTUS	FAGACEAE	CRUCIFERAE	JUGLANDACEAE	PALMACEAE	BIRCH	MAGNOLIA	MISCELLANEOUS	PLATES EXPOSED	TOTAL AVERAGE PLATE COUNT	WIND	WEATHER CON DITIONS
1927																						
Jan	4	13					1											8	14	W	LF*	
"	10	24																1	24	SW		
"	12	3	7	1										2				8	13	W	Cold	
"	17	1																1	1		Cl *	
"	21	3																6	3	S	Cold	
"	24	2		1														5	3	S	Cold	
"	28	40	1	2			2							2				6	47		Rain	
"	31	6																3	6		Rain	
Feb	4	7	1															6	8	S		
"	7	6	1											1				6	8		Clear	
"	10	15	7				2							2				6	26	S	Rain	
"	14	1	1				2							4				4	8	N	Rain	
"	24	3	1				1											6	5		Rain	
"	26	10				16	20							16				1	62	S		
Mar	1	1					3											6	4		Cl	
"	3	1					2											1	3		Rain	
"	7	15				6	1	55										5	77	S	Rain	
"	10	6					1											6	7		Cold	
"	15	5					1	8										5	14	N		
"	18	4	6			171	1	4					1					6	187	N		
"	22	1					1	1					1					6	4	N		
"	24	4					1	1					1					6	7	S		
"	28	14					1	2										6	17	N		
"	31						4	4										1	8	N	Rain	
Apr	5	19	1			2	1				3							6	26		Cold	
"	7	39	8			8	4	3			8	1	1					6	33	N	Rain	
"	15	1	6				1				5	1						5	16		HF*	
"	18	3	4			2	1				6		2					5	18	S		
"	22	12	10			3	1				9							6	35	S		
"	25	10	17			3	2				6				4			6	42	N		
"	28	41	31			2	1				7							6	82	W		
May	2	25	16			1	2				4							6	48	W		
"	5	9	9				1				2							6	21	N	Rain	
"	17	26	7			1	3										3	8	40	N		
1926																						
May	20	94	10			1	1	2										2	9	111	N	
"	24	54	3			1	1	1										1	8	61	N	Cold
"	27	78	13			1	1	4		1								2	9	100	N	
June	1	53	9				1	2										1	8	66	W	
"	3	52	7			1	1	3	1									1	9	66	S	Cold
"	7	16	3			1	1	2	1									1	9	25	S	Cl
"	10	33	9			1	1	1										1	7	46		Cl
"	14	24	3			1	1	2										1	9	32	N	
"	17	12	2		1	1	1	2										1	9	20	W	Cold
"	21	12	4			1	1	1			1							2	10	22	N	
"	24	16	5			1		1			1							1	8	25	S	Cl
"	28	8	1					1										1	9	12	N	
July	1	6	1		8			1										2	9	18	N	
"	6	8		1				1										8	10	W	Cold	
"	8	3						1			1							9	5	W	HF	
"	12	3		1	1													6	5	W		

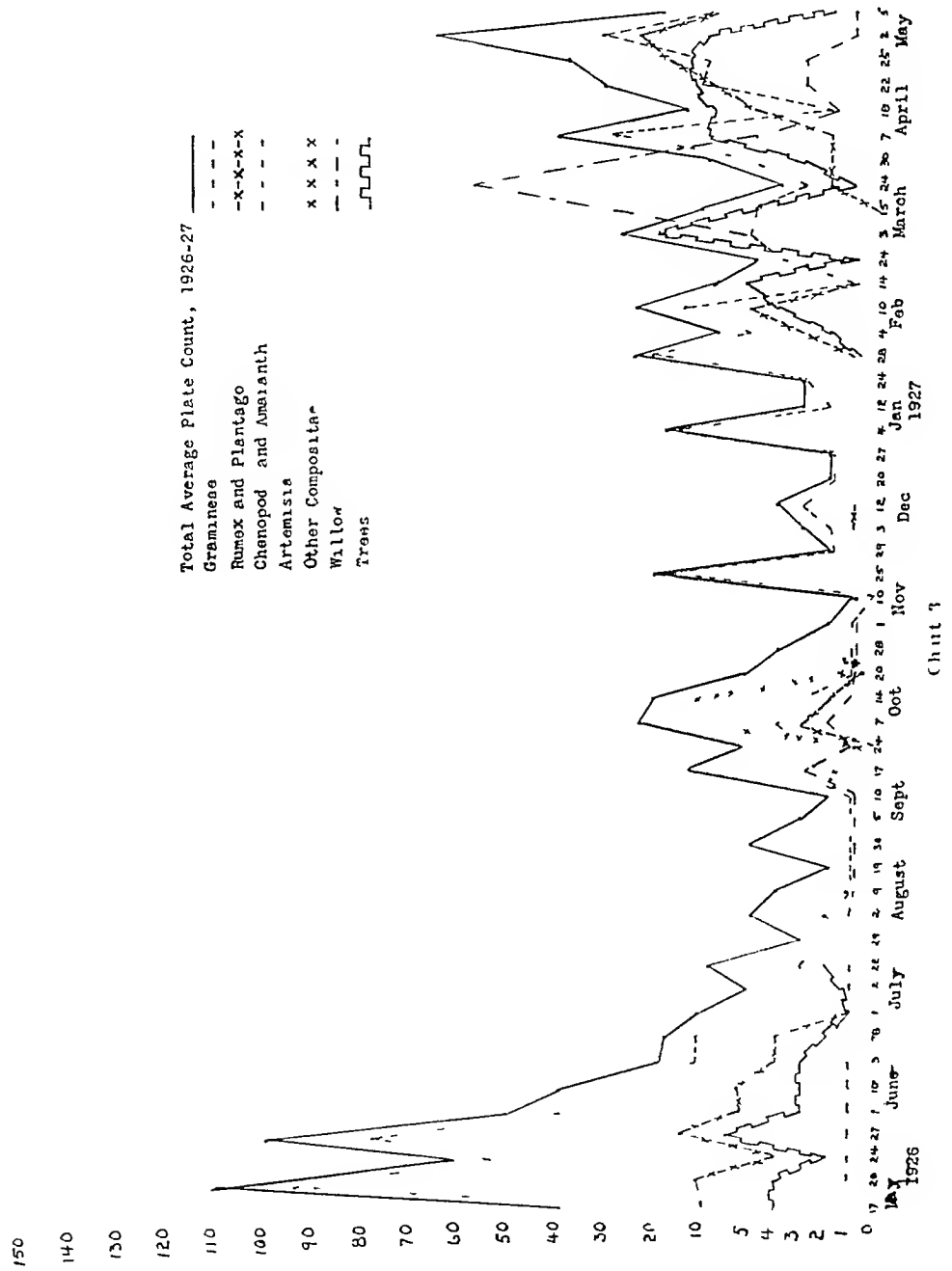
*LF—Low Fog HF—High Fog Cl—Cloudy

TABLE II—CONT D

		GRAMINEAE	RUMEX AND PLANTAGO	ARTEMISIA	OTHER COM POSITAE	TEA MAYS	AMARANTH AND CHENOPOD	SALIX (WILLOW)	ACACIA	FINACEAE	TYPHA	EUCALYPTUS	FAGACEAE	CRUCIFERAE	JUGLANDACEAE	PALMACEAE	BIRCH	MAGNOLIA	MISCELLANEOUS	PLATES EXPOSED	TOTAL AVERAGE PLATE COUNT	WIND	WEATHER CON DITIONS
July	15	4					1			1				1					1 10	8	W		
"	19	4		5			1			1									1 10	13	W		
"	22	2			1	1	1												9	5	W		
"	26	2		1						1									9	4	W		
"	29	2		1															10	3	W		
Aug	2	2		1			1												10	4	W	HF*	
"	5	2		1			1												11	4	W	HF	
"	9	3		3															9	6	W	HF	
"	12	2		1			1												9	4	W	HF	
"	17	1																	9	1	S	HF	
"	19	1		2	1		1												8	5	W	HF	
"	23																		9	0	W	HF	
"	26	1		1			1			1									10	4	NW	Rain	
"	30	2		3			2			1	1								8	9	W		
Sept	2	1		1	1					1	1								1 10	6	W	HF	
"	7	1		1			1												10	3	W		
"	10	1		1			1												7	5	W	HF	
"	13	1					1												8	2	W	HF	
"	17	5		2	4		2											1	2	14	N		
"	21	3		4	2		2												1 6	3	18	S	
"	23	2		1															1	4	6	S	
"	24	1		1			1												4	2	7	N	
"	25																		1	0	W		
"	27	9		1	1		1			1									3	13	W		
"	30	3		1	2		2												1	4	9	W	Rain
Oct	3	2	3	2	12		3			1									1	24	S		
"	7	7	3	2	13		2			2	2								7	27	S		
"	10	5	3		23		1								7		1		7	40	SW		
"	14	1	1	1	1					1	1								7	6	S		
"	18	2	2	1	1														7	6		HF	
"	21	1	1	1			1			1									8	5	SW		
"	25	1		2	1		1												7	5	SW		
"	27	3								1									1	4	N		
"	28	1		1	1		1												7	4	SW		
Nov	1	1	1	1	1					1									7	5	NW		
"	4	2		1	1		1			1									8	6		LF	
"	8	1	1	1	1					1									8	5	N		
"	11	1	1	1	1		1			1									7	5		Rain	
"	15	1	1	1			1												7	4	NE		
"	18			1															3	1		LF	
"	22	42																	1	42	W		
"	25	1					1												3	2	S	Rain	
"	29	2		1															3	3		Rain	
Dec	3	1	1	1	1														7	4	N		
"	6	2	1							1									1	6	5	W	
"	7	3	2																2	5	N		
"	8	2	1	1						1									1	3	6	HF	
"	12	2	1	1															7	4	N		
"	15	1	1							1									7	3	SW		
"	20	3								1									8	4	N		
"	27	2																	8	2	W	Pain	

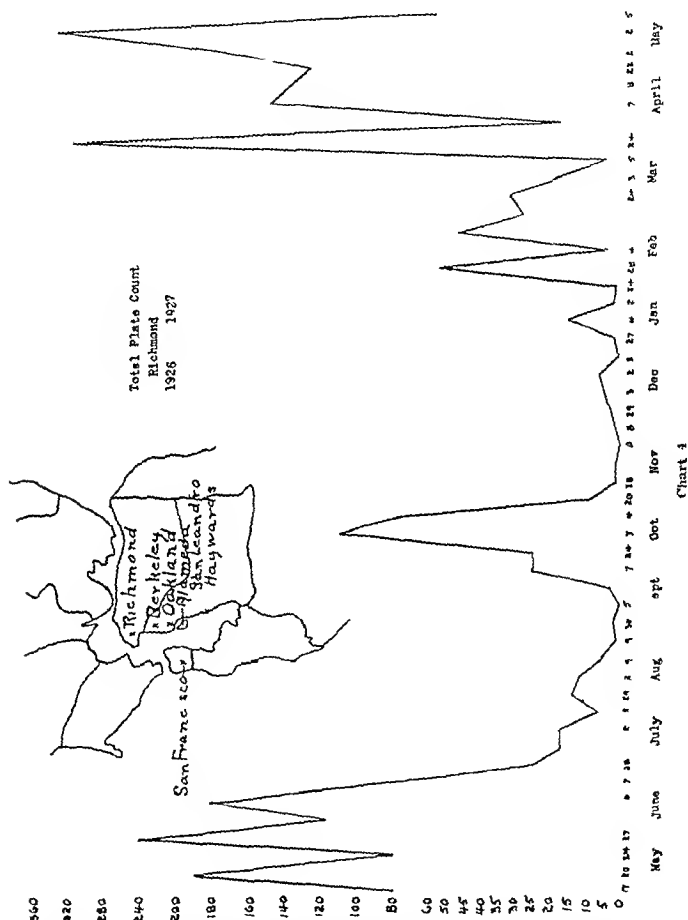
LF—Low Fog HF—High Fog CI—Clouds

pollen likewise was not abundant, though present in the an during July, August, September, and October Pollen of other Compositae, which was probably largely Ambrosia pollen, was found in the last week in September



and during October Franseeria pollen from field observations would constitute a considerable part of the Compositae pollen in those districts shown in Table V, to contain Franseeria Bipinnatifida The possibility must be kept in

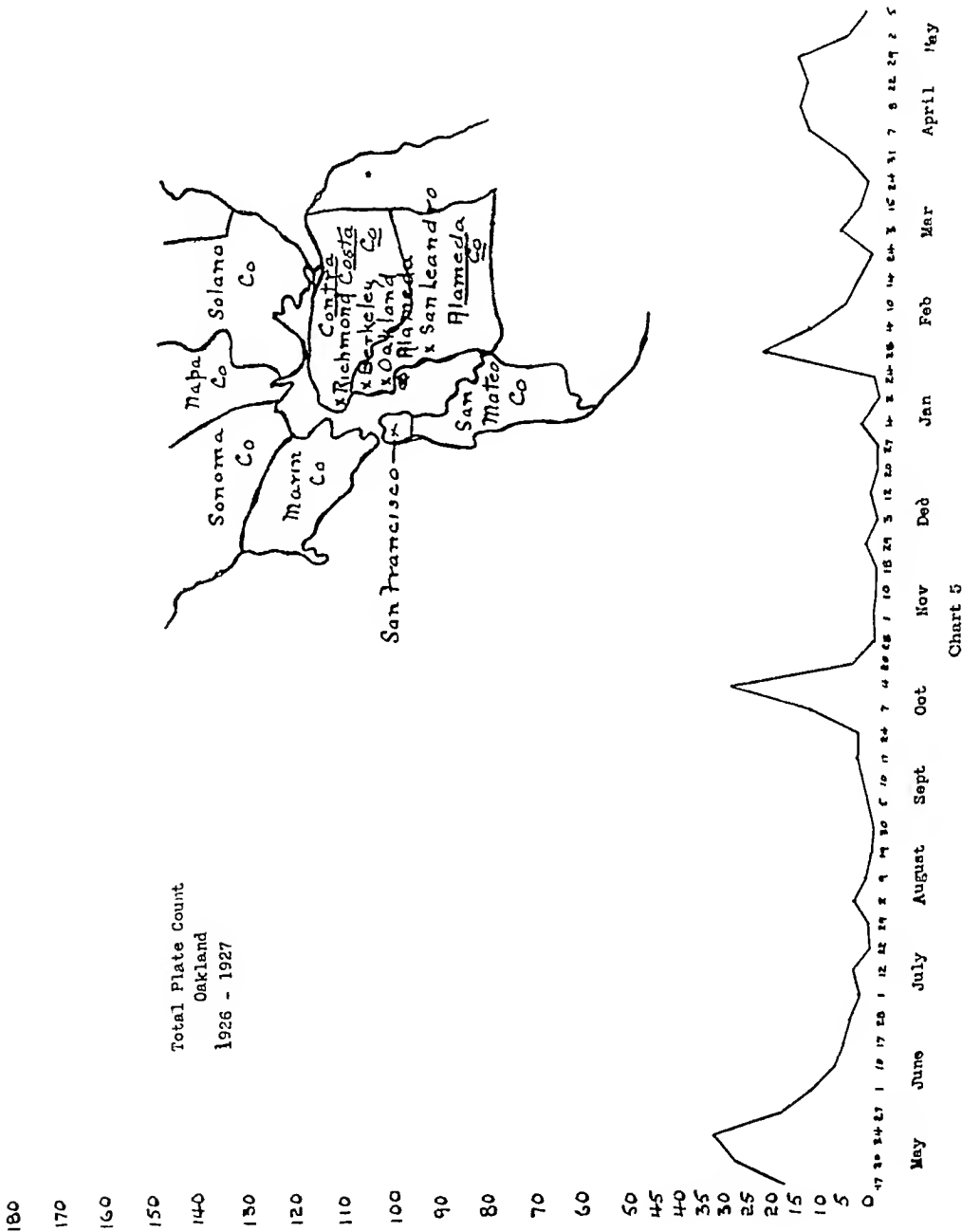
mind that pollen of many species growing at a distance from this Last Bay region may be brought into the air through the action of upper air currents. This possibility has not been studied but seems unlikely because of the nar



low district under consideration, extending from the bay line to the high eastern barrier of hills, the elevation of which is between 1000 and 2000 feet, and because of the prevailing westerly or southwesterly winds. Moreover,

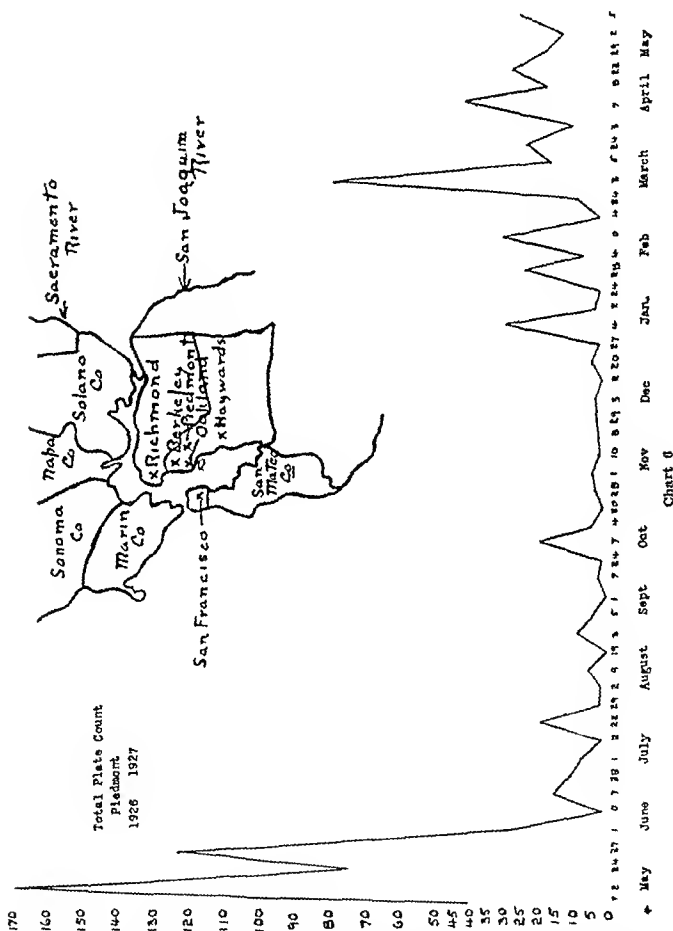
with the occasional hard dry north winds, it was found that the character and amount of atmospheric pollen did not change in any definite manner

Four separate charts of total average pollen counts for each week



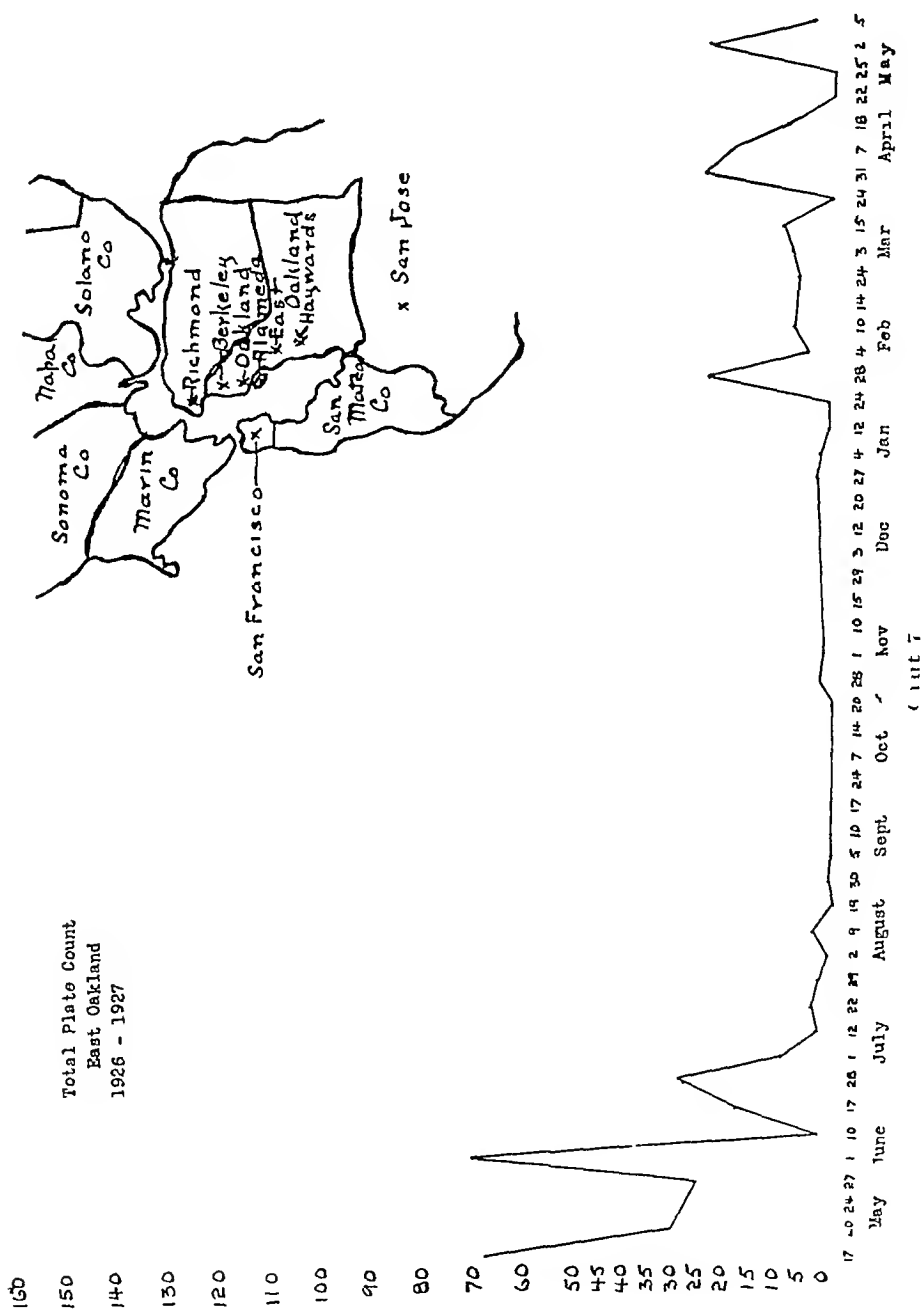
throughout the year are presented in Charts 4, 5, 6, and 7, for Richmond, Oakland, Piedmont, and East Oakland respectively The individual groups of pollens, as shown in Chart 3, were determined in the case of each district

The mass of data obtained does not permit of tabulation in this article, and only charts of total pollen counts are presented. The location of each district with reference to the territory around San Francisco Bay is shown in



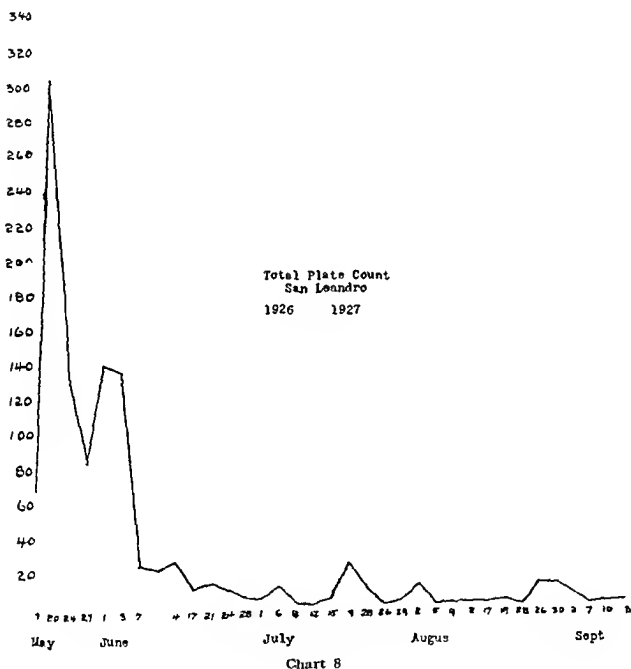
the regional map on each chart. These charts show that Richmond, which has large tracts of vacant land and is subject to much wind, has the highest counts. The count of 308 in March was largely due to willow pollen and about 50 per

cent of the count of 322 in May was due to grass pollen and 50 per cent to *Rumex* and *Plantago* pollens. The peak of 115 in October was due to *Compositae* pollen, which was largely *Ambrosia* rather than *Franseria* or other



Compositae pollen, as determined by field studies. The chart of Oakland shows a comparatively small amount of atmospheric pollen throughout the year. The peaks correspond to those in Chart 3, and the constituents of the

total curve in this, as well as in the other charts, in a general way corresponded to the average values shown in Chart 3. The chart of Piedmont shows higher counts than those obtained in Oakland. The general conclusions in regard to Chart 3 apply to the curve of this region. Piedmont is a small residential district with a population of about 7000 and is situated on the hills adjoining Oakland. It contains more open fields, gardens, and lawns than does Oakland, and this undoubtedly accounts for the higher pollen counts. The station in East Oakland was on a hilly section with little vacant land where the prevailing wind was over Oakland and off the Bay. A total curve in a station on



the outskirts of East Oakland near San Leandro, obtained from May to October is given in Chart 8, and shows a high peak similar to those obtained in Richmond. The different types of curves which have been obtained through out this district demonstrate that a study of the immediate locality in regard to the abundance of the hay fever plants and to the prevailing winds is of great importance in the determination of proper therapy.

This fact is again emphasized by the very low pollen curve in Chart 9 obtained in central Alameda which is a residential district immediately on the bay and with a prevailing wind off the bay. Again a curve obtained near

make such treatment important. The value of a botanic survey of the patient's immediate neighborhood is therefore quite evident, especially in the treatment of refractory pollen-sensitive patients.

7 The definite hay fever and asthma in patients of this district caused by the pollen of weeds, especially of *Artemisia* and *Ambrosia*, well emphasize the fact that small amounts of pollen will produce definite allergic symptoms. When from 1 to 5 pollen grains are found in one square inch of plate surface, the patient, in breathing many cubic yards of air, as he does every day, inhales many times this number of pollen grains. However, the number is small in comparison to the maximum counts of *Ambrosia* pollen reported by Koessler—approximately 900 as compared to our average of 12 to the square inch. This, of course, accounts for the minor significance of our hay fever problem in the fall. I have found many people suffering with mild head congestion, catarrh or bronchitis, and mild hay fever and especially with definite asthma, due to sensitization to fall pollen, instead of the very severe asthma and hay fever encountered in the East and Middle West. Our local problem is likewise different from that of the interior valleys of California, according to my study and treatment of a large number of patients from those regions. The pollens of *Ambrosia*, *Psilostachya* and the *Artemisias* and *Chenopods* are undoubtedly just as productive of sensitization as is the pollen of giant or dwarf ragweed and would cause just as severe symptoms were they found in the air in as large amounts as is similar pollen in the middle western and eastern states.

8 Studies of pollen plates exposed at different levels on the eight teen story City Hall in Oakland confirm Koessler and Durham's conclusion that various species of pollen are evenly distributed throughout the entire air. Table III shows that pollen counts on the average did not vary much on any of the four shades of my own home, emphasizing the even distribution of pollen in the entire air.

BOTANIC SURVEY OF THE EAST BAY REGION OF SAN FRANCISCO BAY IN RELATION TO HAY FEVER AND ASTHMA

My interest in the botanic flora of California, and especially of the East Bay district, began nine years ago when Dr. Grant Selfridge^{6, 7} published surveys made of various parts of California by Professor H. M. Hall^{8, 7} of the University of California. A survey of the East Bay territory was prepared for me at that time by Professor Hall, and since then I have made constant observations on the flora of all parts of this district, particularly in regard to abundance of distribution and time of pollination of the various species of hay fever-producing plants. During the last year these observations have been extended by an intensive study of this region by a botanist, Miss Weisendanger, and the assembled data are presented in Table V. Professor Hall has checked over our data in regard to frequency of distribution and duration of pollination of the various species included in this survey, for which help I am deeply grateful. The separate districts studied were visited many times during the year to check as accurately as possible on the data presented. The data presented in Table V are arranged in the same manner as those in the

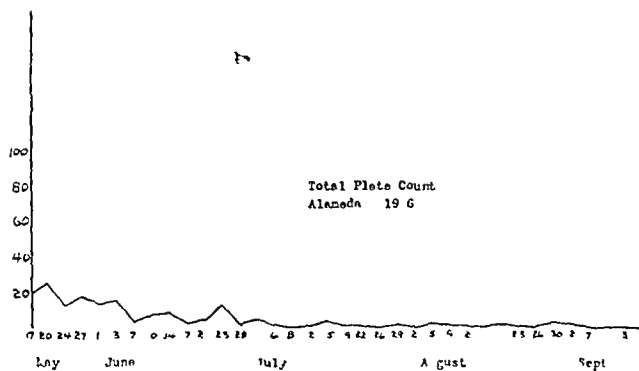


Chart 9

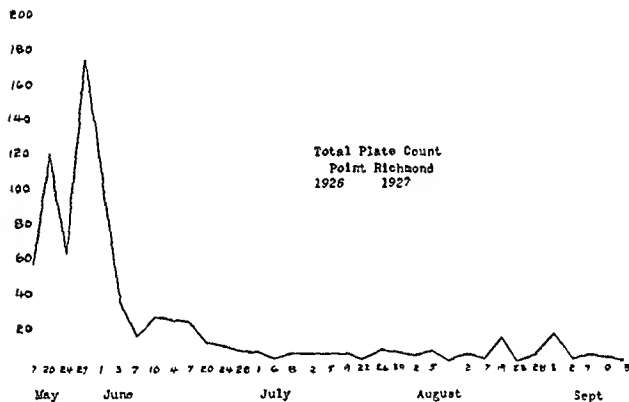


Chart 10

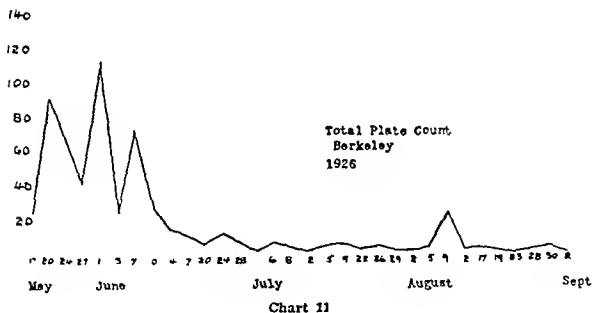


Chart 11

make such treatment important. The value of a botanic survey of the patient's immediate neighborhood is therefore quite evident, especially in the treatment of refractory pollen-sensitive patients.

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BOTANIC SURVEY OF THE EAST BAY REGION OF SAN FRANCISCO BAY IN RELATION TO HAY FEVER AND ASTHMA

My interest in the botanic flora of California, and especially of the East Bay district, began nine years ago when Dr. Grant Selfridge⁶ ⁷ published surveys made of various parts of California by Professor H. M. Hall⁷ of the University of California. A survey of the East Bay territory was prepared for me at that time by Professor Hall, and since then I have made constant observations on the flora of all parts of this district, particularly in regard to abundance of distribution and time of pollination of the various species of hay fever-producing plants. During the last year these observations have been extended by an intensive study of this region by a botanist, Miss Weisendanger, and the assembled data are presented in Table V. Professor Hall has checked over our data in regard to frequency of distribution and duration of pollination of the various species included in this survey, for which help I am deeply grateful. The separate districts studied were visited many times during the year to check as accurately as possible on the data presented. The data presented in Table V are arranged in the same manner as those in the

TABLE III
TOTAL POLLEN COUNTS ON VARIOUS EXPOSURES OF A HOUSE IN PIEDMONT

1926	EAST	WEST	NORTH	SOUTH	1927	EAST	WEST	NORTH	SOUTH
Aug 29			2		Jan 4	33	7	20	17
“ 30			7		“ 13	—	15	—	1
“ 31		4	—		“ 17	1	3	—	—
Sept 2		1	—		“ 21	5	5	—	—
“ 7		—	5		“ 24	1	—	—	—
“ 10		1	—		“ 26	20	25	—	—
“ 11		1	—		Feb 4	6	10	—	—
“ 21		—	—		“ 7	12	7	—	—
“ 23		—	—		“ 10	52	8	—	—
“ 24		3	11		“ 14	—	5	—	—
“ 25		—	—		“ 24	6	13	—	—
“ 30		1	—		Mar 1	3	7	—	—
Oct 3	20	27	2	3	“ 7	70	253	—	—
“ 7	8	8	2	7	“ 10	13	2	—	—
“ 10	9	7	5	3	“ 15	40	14	—	—
“ 14	2	1	—	3	“ 18	54	42	—	—
“ 18	3	1	3	1	Apr 5	—	54	—	—
“ 21	—	1	—	3	“ 7	5	43	—	—
“ 22	2	1	1	15	“ 15	11	35	—	—
“ 27	4	2	—	1	“ 18	6	19	—	—
Nov 1	1	—	1	—	“ 22	5	49	—	—
“ 4	7	4	1	4	“ 25	—	33	—	—
“ 8	—	—	—	—	“ 28	30	8	—	—
“ 9	—	—	—	13	May 2	9	8	—	—
“ 11	1	—	1	1	“ 5	5	45	—	—
“ 17	8	—	4	3	“ 22	1	2	—	—
Dec 3	2	—	—	—	“ 24	6	8	—	—
“ 6	5	—	—	—	“ 25	20	4	—	—
“ 12	2	1	3	—					
“ 15	—	—	—	—					
“ 20	10	9	—	3					
“ 27	3	1	1	3					

excellent survey of Southern California by Piness Miller and McMinn.⁸ This survey of the east shore of San Francisco Bay adds another study to the sectional ones already published by Scheppegrell,¹⁰ Key,⁹ Watson and Kibler,¹¹ Bernton,¹ Templeton,¹³ Duke and Durham,¹⁴ Kahn,¹ Balyeat,¹⁶ Phillips,¹⁷ and Waring.¹⁸

This table indicates that the trees, except for eucalyptus and pine, pollinate during February, March, and April. The relative abundance of each species is shown for the different districts. The importance of tree pollen in hay fever or asthma depends on the proximity to the given trees, the amount of pollen produced, and on the susceptibility of the patient as indicated by history and skin reactions. This would apply especially to such trees as olive, which are scattered throughout this region and to the pollen of which many patients are markedly sensitive. The same considerations are true of sweetgum and oak, which are relatively more common as indicated in the table of distribution.

The important grasses out of the 43 fairly common species included in Table V can be ascertained by a study of the table. Such an analysis will indicate that the grass pollen which is found in the air throughout the year, as indicated in Chart 3, consists largely of *Poa annua* pollen through the winter months, of *Poa annua*, *Poa pratensis*, *Bromus hordeaceus*, and *Festuca pollens* during March, April, and early May, of *Elymus*, *Phalaris*, and especially *Lolium perenne* in late May and June, of *Lolium perenne* and

TABLE IV

PERCENTAGE OF VARIOUS POLLINS IN THE AIR THROUGHOUT A PERIOD OF ONE YEAR, BASED ON 649 PLATE COUNTS MADE AT FROM FOUR TO TEN STATIONS IN THE EAST BAY DISTRICT

Our Findings (East Bay District)		Dr Kocssler's Findings (Chicago)	
	PER CENT		PER CENT
Graminere	50 0	Ragweeds (4 species)	65 3
Trees—		Grasses	21 4
Acer	2 0%	Chenopod	4 2
Pine	8 0%	Amaranth	2 1
Eucalyptus	0 1%	Dock and Plantain	2 0
Oak	3 0%	Compositae	1 0
Juglandaceae	0 2%	Hop and Hemp	1 0
Palm	2 0%	All others	3 0
Birch	0 4%	Total-----	100 0
Magnolia	0 4%		
Sax	11 0%		
Rumex and Plantago	11 0		
Other Compositae	4 0		
Artemisia	3 0		
Amaranth and Chenopod	2 0		
Zea Mays	0 6		
Cruciferae	0 2		
Typhaceae	0 1		
Miscellaneous	2 0		
Total-----	100 0		

Cynodon Dactylon pollen in July, August, September, and October Local surveys of the patient's immediate environment would be necessary to decide the importance of any other species which might be in unusual abundance and be the cause of symptoms in the individual patient The treatment of the pollen-sensitive patient with grass pollen antigens which specifically desensitize the patient against pollens to which he is exposed, is, in my mind, productive of the best clinical results

The importance of the pollens of various weeds is indicated in Table V Certain pollens, such as Amaranthus retroflexus, are important only when local surveys or pollen plates show that such plants or pollens are common in the patient's living or working vicinity However, if business or pleasure takes the patient through territory where pollens other than those in this table are found, desensitization to these must be carried out Although the Artemisia vulgaris is fairly common throughout this whole district, pollen counts do not show that much pollen is in the air of the residential areas Again Artemisia Californica is abundant all over the hills immediately adjoining, and these hills are gradually being developed into residential tracts Patients sensitive to either Artemisia or Ambrosia pollen, driving through or working in country or hills where such pollen is common, will develop symptoms which will demand treatment with these pollens

Patients giving reactions to Chenopod, Atroplex, Rumex, or Plantago pollens, who have symptoms corresponding with the pollinating season, as indicated in the table, should be desensitized with the proper specific antigens Necessity for Xanthum and Franseia pollen desensitization in this region must be determined by the prevalence of such species in the patient's neighborhood and the severity of skin reactions

TABLE V
THE MOST IMPORTANT HAY FEVER PLANTS OF THE EAST BAY REGION OF CALIFORNIA

NAME		DURATION OF POLLINATION												DISTRIBUTION BY DISTRICTS								
Group I	Scientific Name	Trees Common Name	January	February	March	April	May	June	July	August	September	October	November	December	Numbers indicate relative amounts in each district 1—rare 2—common 3—abundant 4—scarce							
			Each figure 1 represents approximately one week Italic figures indicate period of most profuse pollination												Oakland	Piedmont	Berkeley	Alameda	Richmond	East Oakland	San Leandro	
	[Acer]	Acer	11	1111	1111	1111									10	10	10	10	10	10	10	10
	[Acer saccharum]	White Maple			111	111									1	1	1	1	1	1	1	1
	[Acer negundo]	Box Elder		111	1111	1									1	1	1	1	1	1	1	1
	saccharum	Silver Maple		1111	1111	111									1	1	1	1	1	1	1	1
	Alnus rhombifolia	Alder		111	111										1	1	1	1	1	1	1	1
	Betula alba	White Birch			111	1111									1	1	1	1	1	1	1	1
	Juglans Californica	Calif Black Walnut			11	1111	11								1	1	1	1	1	1	1	1
	[Juglans regia]	Eng Walnut			11	1111	1								1	1	1	1	1	1	1	1
	[Liquidambar orientalis]	Oriental Sweetgum			11	1111									1	1	1	1	1	1	1	1
	[Liquidambar racemosa]	Western Sweetgum			11	1111									1	1	1	1	1	1	1	1
	Populus deltoides	Carolina Poplar		1111	111										1	1	1	1	1	1	1	1
	[Quercus agrifolia]	Coast Live Oak		111	111										1	1	1	1	1	1	1	1
	[Quercus lobata]	Valley Oak		11	1111										1	1	1	1	1	1	1	1
	[Quercus dumosa]	Scrub Oak			1	1111									1	1	1	1	1	1	1	1
	[Quercus kelloggii]	Black Oak			11	1111									1	1	1	1	1	1	1	1
	Salix lasiolepis	Arroyo Willow		11	1111										1	1	1	1	1	1	1	1
	Babingtonia	Weeping		11	1111										1	1	1	1	1	1	1	1
	[Eucalyptus spp.]	Gum Tree						1111	1111	1111	1111	1111	1111	1111	1	1	1	1	1	1	1	1
	[Pinus spp.]	Pine	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1	1	1	1	1	1	1	1
	Olea Europaea	Olive				1111	111								1	1	1	1	1	1	1	1

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PURPURIC SMALLPOX

REVIEW OF RECENT STUDIES*

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PURPURIC smallpox is a rare clinical entity characterized by the total absence or atypical development of the poxles and by a diffuse erythema, and generalized hemorrhagic changes of the body. Its diagnosis is often confusing, and its differentiation from other purpuræ extremely difficult. It is always fatal and constitutes by far the smallest group of malignant smallpox. This, in substance, was the experience of the Minnesota epidemic of 1924-1925.

CASE A 24 447 1—A white man, aged sixty-four years, an orderly in the contagious wards of the hospital, was suddenly taken ill with severe headache and nausea on June 20, 1924. His skin was flushed. Next day he developed severe pain in the right lumbar region and in the upper abdomen which radiated to the right shoulder and to the right groin. On June 22, his face became bright red. His throat was injected. His entire body became covered with a diffuse erythematous rash. There were numerous petechiae and ecchymoses over the abdomen and extremities. Temperature and pulse were normal. Leucocyte count was 11,000. Death occurred on the following day or the fourth day after the onset of illness. No definite clinical diagnosis was made. This occurred at the time when comparatively few cases of smallpox actually existed in Minneapolis and no epidemic was declared in existence in the city. At the coroner's necropsy, aside from the extensive hemorrhagic changes already described and the multiple hemorrhages within the body cavities, no pathology which might account for this sudden termination was found. Death was certified as purpura hemorrhagica, direct cause undetermined.

Some six months later, however, prompted by the experience of the intervening months, a careful microscopic examination of the skin was again made and characteristic early lesions of smallpox was demonstrated.

During the height of the epidemic, not a few patients were seen at the receiving ward of the hospital on whom a diagnosis of measles, scarlet fever, or influenza had been made and who died within from twenty-four to seventy-two hours from purpuric smallpox, indicating that the average practitioner might not be prepared to recognize the condition even during an epidemic.

Similar experiences were reported by Councilman² and Litten³ and more recently by the health authorities of Windsor, Ontario, Detroit, Michigan, and of the Minnesota State Board of Health,⁴ all of which illustrate the diagnostic difficulties of this particular type of smallpox.

Twelve hundred and seventy-six cases of smallpox were reported in Minneapolis during the twelve months ending February 28, 1925, with three hundred and thirty-two deaths, or a rate of 26 per cent.

Four hundred and eighty cases came under our hospital care during the same period with two hundred and nineteen deaths, or a rate of 44 per cent.

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Forty eight cases or 10 per cent of the total number of admissions, were diagnosed as purpuric smallpox all of which died

The present report is based upon the studies of these forty eight clinical cases of purpuric smallpox a large number of the skin lesions being obtained



Fig 1—Petechial spots in purpuric smallpox



Fig 2—Diffuse petechiae ecchymoses and subcutaneous hemorrhages in purpuric smallpox

by postmortem biopsy and five complete necropsies, as well as upon the similar studies of a large number of cases of pustular smallpox

Detailed reports on the clinical and hematologic studies of these 48 cases and on the studies of the blood in over 200 cases of smallpox have already been published elsewhere and are therefore omitted^{5 6}

Two distinct clinical types of hemorrhagic smallpox were recognized, namely, the hemorrhagic pustular type and the purpuric type. The latter was again differentiated into the primary form and the secondary form.

Briefly, the hemorrhagic, pustular type was characterized by hemorrhages into the fully developed pustules of confluent or severe pustular smallpox. The mortality in our series of 131 cases was 80.9 per cent. It was probably a local hemorrhagic reaction to the secondary invasion of hemolytic streptococci into the pox. The organisms were recovered from the lesion, often in pure



Fig. 3—Early variola degeneration of the epidermis massive subcutaneous hemorrhages

culture, in all the cases examined. Typical nonhemorrhagic pustules, on the other hand, were always sterile.

In the purpuric type, the entire clinical phenomena would seem to be explained on the basis of a systemic insult incident with this infection and consisting of a series of true purpuric changes aptly grouped under the term "hemorrhagic diathesis." It was characterized by the conspicuous absence of the pox and by a diffuse erythema of the skin accompanied, sooner or later, by the widely scattered patchy ecchymoses and punctate subcutaneous hemorrhages over the entire body. They appeared in crops, many became

enlarged and coalesced. They showed no particular areas of predilection. The face and neck showed few petechiae or ecchymoses but were often deeply red or described as 'Dusky bluish purple' while the dependent parts showed a deep plum color discoloration (Figs 1 and 2).

Subconjunctival hemorrhages were a constant and early change noted practically in all cases of purpuric smallpox.

In a group designated as the primary form of which there were 26 cases, no other skin manifestations were observed clinically. In another group of 22 cases, identified as the secondary or late form in which the cutaneous discolorations were less conspicuous until late in the course of the disease there were, in addition a varying number of atypical pox scattered over the body, either diffusely or more often in limited areas. These pox were retarded in their development. They were bluish in color, small, flat and



Fig. 4.—Confluent degeneration of the epidermis from Case A-447

slightly umbilicated and contained little exudate. More often they resembled dried vesicles containing little or no fluid. The diffuse purpuric changes took place in the period corresponding to the late vesicular or the early pustular stage, often the case having begun as a usual form of pustular smallpox.

In many instances mortification of the body in certain parts preceded death by many hours, thus accentuated the ghastly appearance of the victim to a degree seldom observed in other diseases.

Histologically the cutaneous lesions were characterized by a diffuse edema in the papillae and the corium and in most instances by massive hemorrhages beneath the epidermis. The capillaries were often greatly dilated and filled with blood. Perivascular infiltration of mononuclear cells was noted but not conspicuous. In several cases the hemorrhages extended deep into the subcutaneous areolar tissue (Fig. 3).

The changes in the epidermis were less constant in many of the sections of the most typical gross lesions. The epidermis lying over the most extensively hemorrhagic corium often appeared normal. In others, changes were so early as to be entirely overlooked. More frequently, they were early but



Fig 5—Epithelial changes in the pharyngeal mucous membrane

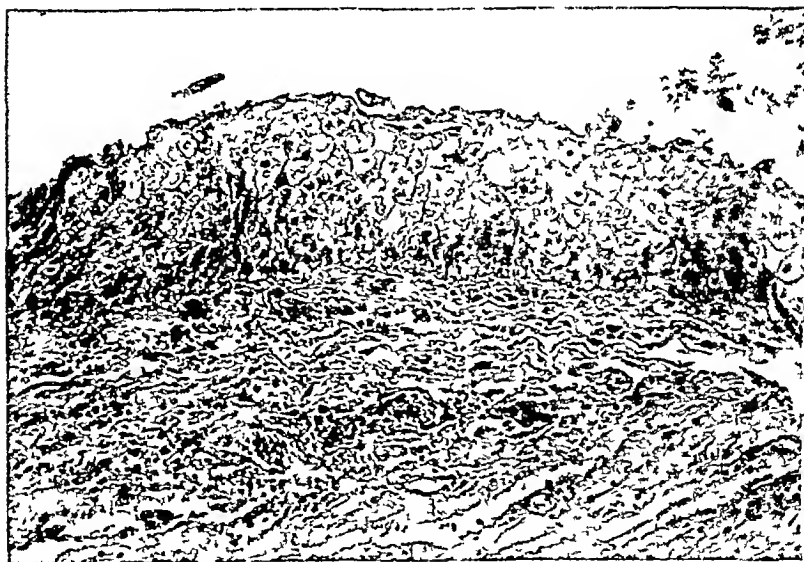


Fig 6—Epithelial changes in the tracheal mucous membrane

sufficiently definite enough to suggest a beginning variola lesion. Again, in a few sections, these changes were so diffuse over a given area as might be designated as confluent (Fig 4). In no instances, except in the secondary form, however, were these lesions in such a stage of development as to be grossly recognized.

A typical epidermal lesion showed merely the early changes of ordinary pustular smallpox. It consisted of the cloudy edematous swelling and the blurring of the outline of the cells of the epithelium, the separation of the cells of the basal layer due to the diffuse edema and areas of the so called ballooning and reticular degenerations of the epithelial cells and in some cases, the more specific early vesicle formation.

The two types of degenerations of the epithelial cells were recognized simultaneously, they occurred side by side. Ballooning degeneration was characterized by the metamorphosis of the cells into a perfectly round vacuole like body with a smooth outline and a darkly staining shrunken, round or distorted nucleus centrally or peripherally located. In reticular



Fig 7—High power reproduction of degenerating and exfoliating glands in the submucosa of the respiratory tract.

degeneration, the nucleus became paler and distorted and the cytoplasm swollen and granular. Coalescence of two three or more of these cells resulted in a large multinucleated cell or a larger trabeculated vacuole containing cellular debris and other degenerative products. These vacuoles were of various sizes. They again coalesced forming an extensive reticulated space in the middle layer of the epithelium which constituted an early vesicle.

Hemorrhagic changes in the interior surfaces of the body were even more pronounced than in the skin obviously because the more delicate mucous membrane was less resistant to the intrinsic degenerative changes as well as to the pressure of the submucosal edema capillary engorgement, and extravasation.

Thus the mucosa of the respiratory tract was uniformly swollen and congested and showed extensive injury to the lining epithelium. Deep punctate hemorrhages or larger ecchymoses were observed along the wall. Microscopically, the cutaneous changes already described were duplicated in substantially the identical manner (Figs 5 and 6) except that the submucosal injuries appeared, as a rule, more intense and further advanced than those of the corium. The epithelium lining the secretory glands beneath the mucosa showed extensive degenerative changes and the cells were freely exfoliated into the lumina (Fig 7).



Fig 8—Epithelial degeneration of the intestine with an area of necrosis

The lungs were greatly edematous and hemorrhagic. The gross appearance closely resembled that of hemorrhagic pneumonia of epidemic influenza. Microscopically, edema in varying degrees and extents and a complete exfoliation of the epithelium in the small bronchi were observed. No cellular infiltrates were noted, but the peribroncheal capillary engorgement and free extravasation were present.

The gastrointestinal tract showed a diffuse edema and congestion throughout. Numerous areas of large ecchymoses in the wall and of submucosal hemorrhages were observed all along the tract. The terminal ileum in one case showed a dark bloody discoloration of the wall throughout its entire thickness while in another the hemorrhages in the colon were more diffuse and confluent, the mucosa was raised in some areas, roughened, and showed a beginning ulceration and sloughing. The lumen contained a large amount of tarry matter. Microscopically, similar degenerative changes of the epi-

thelium leading to necrosis or exfoliation, as well as the diffuse extravasation of blood throughout, were noted (Fig 8)

The heart showed subepicardial and subendocardial hemorrhages. The liver and kidneys also showed hemorrhagic areas beneath the capsules. Microscopically, the liver showed a slight cloudy swelling, and numerous wandering mononuclears between the cords and in the portal spaces (Fig 9). The spleen was tense and the pulp was dark red and firm in consistency. The malpighian corpuscles were not prominent. Hemorrhages were also noted in the mesentery and in the peritoneal wall.

Uterine hemorrhages were noted in all adult females suffering from this type of smallpox. They were interpreted as menstrual by the patient. At

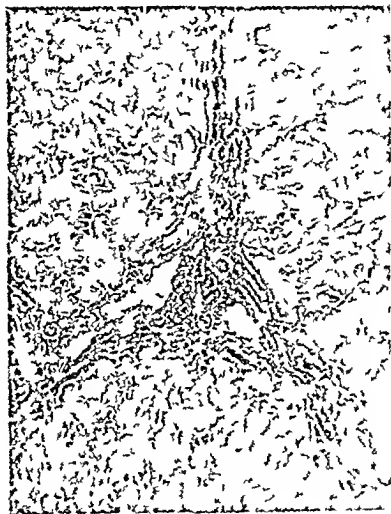


Fig 9—accumulation of lymphocytes in the portal spaces and their infiltration between the cords of liver cells

least in one case a threatened abortion was seriously considered and the patient was treated accordingly in a private hospital. Degeneration and exfoliation of the glandular epithelium of the prostate were also present.

Hemorrhages beneath and within the serous membrane lining the body cavities were quite common.

Hemopericardium was noted in one hemorrhax in another and hemo-peritoneum in the third. Two brains were examined. They showed a superficial venous congestion and a diffuse subarachnoid edema. No gross hemorrhages were noted either in the ventricles or within the substance of the brain.

Hemorrhages occurred in the bone marrow in the shaft of adult femur. The yellow marrow was largely replaced by a semigelatinous blood. Microscopically, the field was flooded with blood and showed islands of cellular elements in which normoblasts predominated but which also contained a few myeloid cells. It showed an undoubted hyperplasia.

That the condition presented an extreme degree of bacteremia was shown by the antemortem demonstration of numerous organisms of the streptococcal group in a simple blood smear obtained from a clean finger tip (Fig 10.)

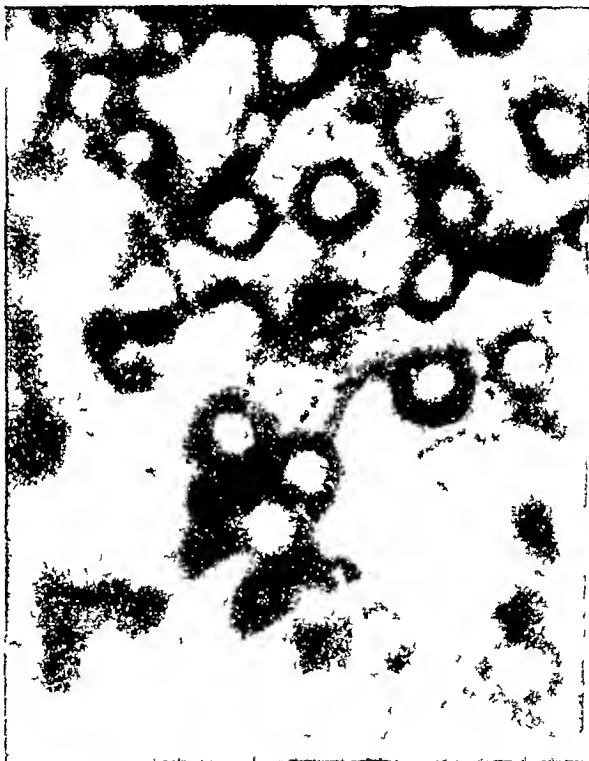


Fig 10—An antemortem blood smear showing streptococci

In one case, direct smears from the cut surfaces of the liver and the spleen, five hours after the death of the patient, showed a large number of streptococci.

Certain characteristic changes observed in the blood of smallpox patients were considered of value, particularly in the prognosis of this disease. In the purpuric type of smallpox, the composite blood picture was not only of a prognostic value, but also by far the most definite and important means at our command of making a diagnosis and differentiation from other purpuric conditions.⁶

The blood findings in purpuric smallpox are briefly summarized as follows:

The Erythrocytes—The erythrocytes showed a normal count and a corresponding hemoglobin percentage except in a few protracted cases of the sec-

ondary form in which a moderate degree of anemia was evident. A large number of normoblasts and a definite degree of polychromatophilia and basophilic granular degeneration were invariably observed in the circulating blood from the very onset of the disease. These changes often preceded the diffuse cutaneous manifestations.

The Leucocytes—Rapid disintegration and disappearance of the mature cells of the myeloid origin were noted. Striking metamorphosis of the mature leucocytes in the circulating blood was observed in every case. This consisted of a condensation of lobes of the segmented nucleus into several separate spherical bodies which gradually diminished in number until there remained only a single round body practically devoid of the cytoplasm. (Fig. 11.)

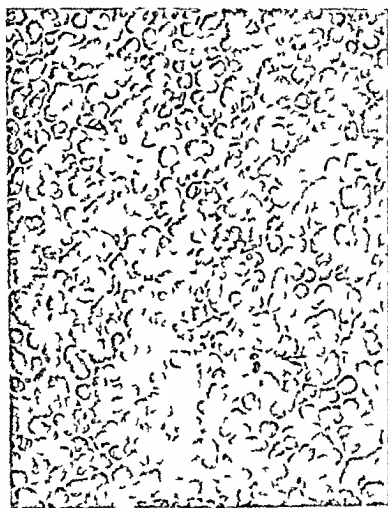


Fig. 11.—Fragmentation and condensation of the nucleus of neutrophilic leucocytes

The Lymphocytes—Rapid decline in the number of polymorphous leucocytes was immediately compensated by a rapid increase in lymphocytes which attained more than 90 per cent of all white cells in spite of a persistent and extreme high total count. Many of the lymphocytes were classed as atypical or immature in that they showed many coarse azurotic granules and basophilic discoloration of the cytoplasm and peculiar lobulation of the nucleus in some or rather loose chromatin structure of the nucleus with nucleoli in others. Apoptosis in some lymphocytes was also observed. In several cases these changes in the lymphocytes were sufficiently characteristic to be taken for an acute lymphatic leucemia.

The Platelets—Unlike in pustular smallpox the initial low platelet count was maintained throughout the course of the disease. It showed a further

decrease until only a few thousands or less were counted in a cubic millimeter. Not a single case was recalled in which the platelet count showed a tendency toward a return to the normal.

These characteristic blood changes were probably by no means specific in the purpuric type of smallpox but rather were interpreted as indicative of a toxemia of the most extreme type, the like of which has seldom been observed in any other disease. This was particularly characterized by the rapid dissolution of the circulating leucocytes, the inhibition of the leucopoietic activity. Diligent studies of the blood in other severe infections and toxic diseases have failed to show the similar alterations.

Practical application of the blood examination in the diagnosis of this type of smallpox and particularly in the differentiation of it from the hemorrhagic pustular type and from acute exanthematous and hemorrhagic diseases, such as scarlet fever, measles, toxic and drug rash, and infectious purpura, was repeatedly found successful during the epidemic when early differential diagnosis of this disease was of the utmost epidemiologic importance.

CONCLUSION

The purpuric type of smallpox presents no anatomic resemblance to the pustular type of this disease. Microscopically, identification may be very difficult in the absence of early variola lesions in the skin.

Diffuse subcutaneous, submucous, and subserous hemorrhages are the most striking feature of this type of small pox. Intense streptococcal bacteremia is demonstrated in practically all of the cases studied.

The blood picture, while probably not specific, is sufficiently characteristic in every case of purpuric smallpox as to be considered of the prime importance in its diagnosis and its differentiation from other purpuras.

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THE PRESENT STATE OF OUR KNOWLEDGE OF GINGIVITIS*

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PURPOSE

THE purpose of this paper is to treat the present state of our knowledge of gingivitis in part as supplied by the literature, which is voluminous and contradictory, and in part by my own experience which now goes back over a period of fifteen years with quite constant observations. During this time I have presented many papers before different societies but have published very little. Our knowledge of the subject has been a gradual development, and while the last word is still distant certain facts have been or should be quite well established. At least enough of these facts may be gathered together so that, when the approach to the subject is suggested by several authors, is fundamentally correct, the main issues will be founded upon certainties, the atmosphere clarified in many respects and those portions of the subject which are still problematical will be more promptly cleared up.

The dentists, until recently having this field practically to themselves, are quite controversial, and many of the leaders are dogmatically and diametrically opposites. They disagree widely in their conceptions on etiology, and various individual theories on treatment are tenaciously held to. Thus, with the leaders and authors disagreeing so violently, often at times vitriolic, the rank and file throw up their hands continue to scale, extract, and use chromic acid.

More recently the medical internist having become convinced of the importance of oral foci and their relationships to metastatic manifestations is demanding more from the dentist than a mere tooth extraction or a complete mouth extraction, in the hope that one or more teeth removed may relieve some recognizable chronic infection. With this added stimulus the laboratory has taken a real interest, and now that it is possible to have the combined opinions of the dentist, the internist, and the clinical pathologist some light is gradually dawning. I would like especially to thank my coworker, Dr. A. B. Vastine, a dentist and M.D., whose idealism, vision and enthusiasm are second to none and whose inspiration during the past eight years has been a great stimulus.

The purpose of this paper aims further, to discuss the clinical and pathologic types of gingivitis with methods of study and a standardization for reports. A report on five thousand cases with the bacteriologic findings of over four thousand either by smear, dark field culture or section is included. An attempt has been made to show the relationship of various etiologic fac-

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tors with the position and importance of each, as applied to the etiology of gingivitis and its pathologic reactions. Finally some discussion on treatment has been included, rather reluctantly, because in this our greatest advances must be made in the future.

THE PROBLEM

The term "pyorrhea" we have absolutely discarded, as it is misleading. The minute one speaks of pyorrhea, there is an immediate picture of gums full of pus and teeth falling out. It is just as logical to call only those cases of tuberculosis showing advanced cavity, tuberculosis and disregard all miliary types, as it is to speak only of pyorrhea or consumption of the gums, when this is an end stage of much developmental, forerunning pathology. For the same reasons we have discarded the term "Vincent's angina," because it has been so closely associated with so-called "trench mouth" that it cannot be pictured as anything else. All changes in the gums and investing structures of the teeth of an inflammatory nature we have designated under the general term "gingivitis." We should like to insist on this, since the complication of terms, as suggested by some writers, is not only cumbersome but misleading. The term gingivitis may be qualified to fit the pathology of any given case, as acute ulcerative, chronic suppurative, chronic recessive, and so forth.

Gingivitis may be defined as an acute, subacute, and chronic inflammatory reaction in the gums, and soft, teeth investitures, progressing unless checked to a periostitis and osteitis. It is characterized pathologically, and objectively with variations, by redness, edema, swelling, surface granularity, ulceration, suppuration, and the establishment of deep gingival sulci. Terminally, loss of teeth ensues. It is characterized *subjectively often by nothing*, at times by extreme pain and tenderness in the gums and jaws, with teeth grinding, and more often by bleeding, bad taste, and foul breath. Indeed, many of the objectionable breaths are due to the gaseous production of gas-forming cocci in gingival pockets. *Thus gingivitis, all forms of inflammatory reactions in the gums, is inflammatory and as such is infectious in origin.* Gingivitis is present, in confirmation of some, in spite of other, widely advertised dental creams and powders, in about 90 per cent of our population in some form or other. This does not mean that 90 per cent of us have pus pockets or are losing our teeth, but it does mean that we have sufficient potential to reach this end-condition, given time enough.

The general problem of gingivitis is closely related to focal infection, and this paper should be considered in conjunction with my views as stated in a companion presentation.¹ Gingivitis, acting as a composite focus of infection or portal of entry for microorganisms of a virulent or low grade character, is one of the most important and frequent sources of infection in the body protective mechanism. The early recognition is thus of added importance, and its complete control one of the greatest prophylactic and preventive situations confronting us today. With the early recognition, eradication, and control of gingivitis, a great many of the chronic manifestations of midlife and later may be forestalled.

HISTORICAL

Piorrhoea, gingivitis, stomatitis, subperiosteal periostitis with subgingivitis and what not, have been discussed in the literature for a century. I feel that the work of Dr. Allen J. Smith in conjunction with M. F. Barrett and reported by the latter in 1914, suggesting the role of endameba as an etiologic factor, followed by Bass and Johns and later by the experimental work on focal infection, especially of Rosenow, has done a great deal to focus attention on and advance the general problems of gingivitis. Barrett reported an endameba in all of forty six cases of suppurative gingivitis, most of which were advanced cases obtained from the wards of Old Bloedel and while it was not offered as a sole etiologic factor it was strongly suspected, and emetine locally was advised. Lass and Johns² in November 1914 were more emphatic as to the etiologic role of endameba (55 out of 57 cases) and offered emetine hypodermically as a remedy. Later they published a book on the subject. At the 1914 Christmas meeting of the American Society of Bacteriologists, Smith and Barrett⁴ offered a discussion of various endameba described in the mouth up until that time and definitely suggested *Endameba gingivalis* (Gros) as the type which is now held. Kofoid and Swezy in 1923 again studied this protozoa in its relation to *E. dysenteriae* and determined *E. gingivalis* (Gros) a distinct type from the latter. This difference was presented by Smith and Barrett⁴ June, 1915. We are not so sure that *E. gingivalis* has not a definite relationship to some intestinal endameba thought to have little pathogenicity, and more work is required on this phase of the subject.

Discussion started, war raged and while not quite so acrimonious at this time continues nevertheless but on a friendly debatable ground. As an illustration, in 1920 I read a paper before the Society of Bacteriologists and Pathologists in Washington and received a short curt discussion with the statement that in a series of normal mouths in New York school children endameba had been found in a fairly large proportion and therefore it was not to be considered pathologic or pathogenic. This closed the whole subject stat there and then, showing the trend of thought at that time. I never published that paper. In 1921⁶ a preliminary report of two hundred cases was published and this paper is a follow up of that earlier work.

Hunter, in 1780 emphasized the fact that the seat of trouble was in the bone. Torae in 1839 first used the term *piorrhoea* which I think has had its centennial and should be relegated to the archives in favor of the more inclusive term 'gingivitis'.

In a paper at the Annual Symposium of the American Stomatologic Association in 1926 Asgis speaks of the approach to the study of this disease influencing the writing of various authors mostly along the lines in which their conceptions of etiology bear and in reviewing the literature this is especially true. Asgis includes in his paper forty nine references developing the subject with forty nine points of view.

Gottlieb has conceptions of pathology with which I cannot agree,⁸ but he has called attention in the formation of the anatomic ditch at the union of the soft structures of the gum and the tooth to the fact that the detachment

normally never reaches the cement-enamel junction,' but is always above it. Thus I think is most important as an objective sign in determining clinically the presence of gingivitis.

Many and varied reports have appeared on findings of Vincent's spirillum and *B. fusiformis* by smear, but most of these, while advocating them as possible etiologic factors, associate them with definite 'trench mouth' and stomatitis. Burns¹⁰ suggests their significance as precursors of pyorrhea and as possible causative agents in such unknown etiologies as anemia.

The work of Rosenow, following his association with Billings, has done much to establish dental foci of infection. His work has dealt mostly with the tooth and its periapical relationship. I hope to show in this paper that all the principles of that work may be applied to gingivitis and that, in proportion, gingivitis per se is many more times a portal of entry primarily for microorganisms. Thus, it is an even greater potential focus of infection than the periapical sources which are not to be minimized in the least in importance.

In the *Medical Journal and Record*¹¹ a report of the 1926 Symposium on Stomatology reviews the opinion of France, Italy, Poland, Hungary, Germany, England, and America and gives a cross-section of about where the world stands today on gingivitis.

It is quite impossible for me to review completely this literature in this paper, except to call attention to what seems to me to be high lights in my conception of the approach to the problem. In the studies on etiology some speak of pyorrhea as a manifestation of "loss of local resistance" due to systemic diseases, others as the etiology of those systemic diseases, some attempt a relationship to vitamins and food deficiencies, while others assume a rôle of glandular inactivity. Smoking, gingival trauma in mastication, food debris, toxins, mouth breathers, plates and dentures, bridges and crowns, salivary inactivity, acid mouth, abnormal calcium phosphates in the saliva producing calculi and tartar, all have then important adherents. Infection as a causative factor is stressed by only a few authors but mentioned by many. When it comes to treatment, the widest variations prevail, from the occasional paper on vaccines condemned by Krumwiede and Pratt in 1915, through papers on general and local measures, to the radical surgery advocated by Neumann.

A research group at the University of California organized in 1921, of which Beckwith and Simonton¹² have published several papers, has developed along the lines which our own work has taken more nearly than any other group so far as I know. In a recent progress report, however, the conclusion is drawn that "then accumulating data appears to indicate that pyorrhea belongs in the category of nutritional diseases * * *. This does not preclude the possibility of the clinical pictures being often and largely dominated by an infectious process * * * superimposed on an initial predisposition of nutritional origin." As the present paper will develop, I have been led to an entirely opposite conclusion, and I hope it is not due to a too persistent attachment to our avenue of approach.

I have said that gingivitis is a focus of infection, and in a recent paper have outlined these conceptions.¹ The importance of this bears repeating

The gingiva, the seat of inflammation, offers one of the best multiple portals of entry for such microorganisms as the streptococcus to be picked up by the subgingival lymphatics and blood vessels for distribution throughout the body that I know of. In this sense both as portals of entry and as potential focal sources the gingival position is the *most important* in the study of focal infection and control of distant manifestations such as rheumatism, myocarditis, nephritis, arthritis and so forth. Beckwith, et al,¹³ have reported with photomicrographs the proof of the presence of organisms in the subgingival lymphatics, and I have seen them many times the same tissue being controlled by cultures. Thus it may be an established fact that microorganisms admitted through pathologic gingivitis establish themselves in the subgingival tissue where they act as foci of infection fulfilling two three, and four of my conceptions as foci.¹

GINGIVITIS CLINICALLY AND PATHOLOGICALLY

A discussion of what constitutes the normal gingiva and its relationship to the teeth appears in a previous paper.¹ In this paper I have also gone into some detail as to just what gingivitis is clinically and pathologically. Gingivitis divides itself into acute subacute and chronic forms based upon its pathologic reactions associated with its clinical course. This classification is influenced by local variations and etiologic gradations only as those are modified in each case after careful clinical and laboratory study. These gradations, modifications, and variations are not only important for properly fixing the type of case for study and teaching but are most important to the individual patient in outlining treatment. Our fundamental conception of gingivitis not only includes the terminal suppurating pus pockets which any body can see but all the minor degrees of inflammatory changes involving the gums which are now almost universally passed up as normal. Gingivitis is a progressive disease with very small beginnings and it is only its terminal stage which is the well recognized condition now known as pyorrhea.

Gingivitis then, is a nonspecific inflammation of the gums produced by a variety of infecting organisms starting acutely or more commonly insiduously and becoming tenaciously chronic. At first the gingivae show nothing more than redness on pressure with a little granularity on the surface and here and there a shallow increase in the anatomic trenches (this stage of the disease is almost always passed over as perfectly normal). The redness signifying gingival reaction continues and a little exudate appears in the trenches. This is often found only after careful search and sometimes in a clean mouth only at the occlusal surfaces of molars or about a crown. Nevertheless, it is there as a starting point and *given time* will eventually involve the gingivae of all of the teeth. This infection redness and exudation goes on to suppuration ulceration and deepening of the trenches, and well marked pockets are formed about one two or all the teeth. At this stage ridging of the gums (granulative subgingival fibrosis) occurs with atrophy and recession from the teeth resulting in exposure of dentine. During this progression the periosteal investiture becomes involved in many cases eventually terminating in some form of osteitis. I am firmly convinced that

many, not all, of the so-called periapical reaction cysts and periapical pathology without cyst were originally of gingival origin and progression and had little or nothing to do with root canals

This condition attacks all ages and both sexes. I have frequently seen it in very young children, and Dr. Vastine now has a young girl under treatment, the eruption of whose second teeth is markedly interfered with by nothing more than a severe infectious gingivitis. This is yielding very nicely to treatment. The acute and subacute stages are by far most commonly seen in young adult life with slow progression on to middle life, when ulceration and more advanced changes are to be looked for. The very advanced chronic cases which are seen now and then in young life are much more frequently in the forty to fifty decade and about the time most of the teeth are lost for one reason or another. It is to the prevention of this great sacrifice of teeth that our future work must lead us, and indeed we have the means at hand now to accomplish this except that it is somewhat complicated. Neglected and dirty mouths are more apt to have advanced changes, but not in every case. Some of the apparently dirty mouths will be relatively free from infection and advanced gingival changes, while, vice versa, some of the cleanest mouths in which oral hygiene is religiously carried out will harbor virulent, tenacious infection leading to advanced gingival changes. Every case is a careful study unto itself, as to history, symptomatology, clinical appearance, distant manifestations, types of and quantitative infection present and individual reaction to treatment. Gingivitis is an entity only in so far as the gums are involved, it is complex in its course, appearance, etiology, and treatment, and as soon as this complexity is understood and accepted, the tangle of the problem presented today will be decidedly cleared up. The recognition, course, appearance, and etiology are relatively simple, but the treatment is much more baffling. This, however, relying on the correctness of the other studies, is now well advanced when certain procedures are carried out and bids fair soon to be completely straightened out.

REPORT ON 5,000 CASES OF GINGIVITIS

This paper includes the report of 4,347 individual cases where the gingivae have been examined bacteriologically by smear, dark-field, culture, or section. These cases do not belong to a group survey but are the routine cases carefully studied presenting themselves over a period of years. All have had smears, a few dark-fields, many cultures, and a few sections. With cases not included in this group, we have seen well over five thousand which we believe should be a number large enough from which conclusions may be drawn. Statistics mean very little ordinarily, but in large groups they at least point to facts. The same relative methods have been used throughout the study, but necessarily experience has brought improvements, and I desire to offer a standardized method for the examination of all gingival cases. This examination, where possible, should be carried out by one person in the laboratory who will have acquired a uniform gradation of amounts. This is quite important in recommending treatment, since the success of the latter depends almost entirely upon the accuracy of the former. If the patient is taken into the

confidence, as he should be in order to gain his cooperation the improvement as measured by gradation is a good aid to his morale

The mouth is surveyed generally, and the physical condition briefly noted. In the group study of oral foci of infection this first examination should be quite complete and while it helps to have the report of the laryngologist and dentist, the examination should not be confined entirely to areas which they may have pointed out. Cooperation in this respect helps indeed. It is not necessary to use the dark field for examination routinely, as suggested by some, and after a few hundred cases have been examined for familiarity, the dark field should be reserved for blood culture serologic positive cases where the question of *Treponema pallidum* is raised. I have only seen *Treponema pallidum* once in gingival exudates but my group has not included many active secondary syphilites.

After the entire gingivae have been surveyed expose the gums with a wooden tongue depressor moistened in water (so that it does not stick) and obtain material from all possible sources in the gingival sulci both upper and lower. A 22 gauge chrome wire small loop slightly bent is most suitable. Platinum wire is too soft and instruments are too stiff chrome wire is preferred. Whenever the gingival trench is deep the loop should be worked in and out until gross material is obtained. I have obtained positive findings on several occasions following a negative report by attention to the detail of obtaining the exudate. This method if carried out gently will hardly annoy the patients, many of whom are nervous expecting to be hurt. It is not necessary to flame the loop between different situations examined, since any transfer from one pocket to another does not mean anything in the mouth. After the first flaming which the patient may see, assure him that the loop is not hot. Care should be taken not to get hemorrhage since red blood cells in large numbers interfere with the subsequent examination of the slide.

Transfer the materials obtained to a large drop of physiologic salt solution 0.85 per cent, on a microscopic slide evenly mix with the loop, and cover with a square cover slip. Water, of course is not to be used. In the summertime the slides do not need to be heated but if the room is cold and it is winter the slide should be gently heated over the top of the Bunsen flame. A warm stage may be used but this is not absolutely essential. The slides however *must not be cold*. Examine under the high dry power 4 mm objective, as a routine twenty fields should be looked at more may be but certainly no less. This will give one a relatively quantitative idea of each group sought and of the preponderance of one over the other. If this quantitative estimation is reported in grades of 1 plus to 4 plus it will be a great help in outlining and carrying out treatments. Where any type is not present we use ND, not demonstrable. One plus means present but only a very few for example one endameba seen in twenty fields or an occasional spirochete or pus cell. 2 plus and 3 plus mean increasing degrees for example one or two endamebae in every two or three fields. 4 plus means large quantities as two or three endamebae in most every field, spirochete by the millions or heavy exudate of pus.

After the slide has been carefully examined in the fresh, the cover slip is gently drawn across the slide, the latter is fixed either by heat or by a mercurial fluid and stained by any method preferred. A good Giemsa stain is perfectly satisfactory, Giemsa, carbol fuchsin weak alone, Wright's stain, Hiss's capsule stain, plain methylene blue, all give good fields. For endameba we prefer a fixation by Schaudinn's mercurial fluid and a stain by Giemsa or non-hematoxylin. It is believed that the fresh smear examined by the 4 mm objective gives the very best examination showing all factors to the best possible advantage. The stained smears help out in critical study, and in using the Giemsa stain, prevailing types of bacteria may be tentatively enumerated, as for example, pneumococcus, streptococcus, *B. coli*, etc.

The important features to be searched for in order of importance are the spirochete, fusiform bacilli, *Endameba gingivalis* (Gies), bacteria as a group, cocci and bacilli motile and nonmotile, pus cells, red blood cells, desquamated epithelium, and mycelial threads under the general term leptotheix. Unusual findings, such as yeasts, protozoa other than the ameba, crystals, debris, food particles should be noted at the end of the report.

Where cultures are made to determine specifically the bacterial flora or to study in detail a predominating organism, loops are obtained directly from the gingival sulci and planted on appropriate media. The media are warmed in the incubator and immediately incubated. As a routine we use rabbit, guinea pig, sheep, or human blood agar plates (very light blood) streak planting, autogenous blood agar plates, autogenous blood the method of Heist, plain broth, sugar broth, ascitic fluid, and anaerobic cultures. The latter are prepared by inoculating a plain agar slant and stab, pouring a tube of plain broth, glucose broth, ascitic fluid, whole blood, or tissue and covering the top with a good paraffin layer. Special medium, of course, may be added for special purposes. It is not essential to culture mouths routinely unless one has a large laboratory force, but in given cases not responding to treatment a chemotherapeutic may be worked out by autogenous cultures*. It is not practical, of course, to section gingivae routinely, but this should always be done after culturing operative bits of tissue. For study, of course, small bits of tissue may be removed by biopsy or taken at autopsy.

Referring to Table I, which represents the fresh smear examinations, it will be found that spirochete appeared 3,778 times or 96.4 per cent, the endameba appeared 2,809 times or 71.6 per cent, the spirochete and endameba were present at the same time 2,668 times or 68.0 per cent. The spirochete appeared alone 1,110 times or 28.2 per cent, the endameba appeared alone 141 times or 3.6 per cent. There were 428 cases which had either no clinical evidence of gingivitis or at least very slight evidence in which neither the spirochete nor the endameba could be demonstrated. These include the control negatives, and there was not a single case of clinical gingivitis in which one or the other of these organisms could not be demonstrated.

The 2,613 or 66.6 per cent of the smears showing bacteria represent those cases having outstanding clumps and masses of bacteria growing in the

*Since this paper was presented I am routinely culturing all cases and I would advise at least a blood agar plate be studied for each gingival flora.

TABLE I
INCIDENCE OF OCCURRENCE OF DIFFERENT ORGANISMS

	NUMBER	PER CENT
Total number of cases	5 000	
Number of cases recorded	4 347	
Number of cases of gingivitis	3,919	90.2
Number of cases of normal	428	9.8
Spironema present	3 778	96.4
Endameba gingivalis present	2 809	71.6
Endameba and spironema present in same case	~ 668	68.0
Pus cells present	3 919	100.0
Bacteria present	~ 611	66.6
Fusiform bacillus present	~ 211	~ 6.4
Spironema present alone	1 110	28.2
Endameba present alone	141	3.6
Leptothrix present	2 082	53.1
Trichomonas present	1	0.02
Cercomonas present	48	1.2
Bilantidium present	2	0.04

gingival sulci. The 33.3 per cent remaining of course were not sterile, but the bacteria were not present at least in smears in sufficient quantities to be noted. Indeed, in smear, normal mouths are surprisingly free from everything. It is frequently very difficult to get even enough to look at. On the other hand, advanced gingivitis has so much that the smears may easily be made too thick to be viewed.

The fusiform bacillus that is a large slightly curved fusiform bacterium was noted 2211 times or 56.4 per cent. For some time we have not regarded the relationship between this bacillus and spironema as a symbiosis but have considered that they have a closer bearing. Their position as regarded will be discussed later on. We believe they should be noted where they are present in the absence of spironema but cannot give any figure on this relationship at this time.

Table II points out the gradations of spironema and endameba based on the standardization previously outlined. In the case of spironema the occurrences of 1 plus 2 plus and 3 plus are about the same while the 4 plus is decidedly lower in number. In the case of endameba there is a marked difference between the occurrence of 1 plus and 4 plus. I think these figures are the result of normal etiologic incidence but I also believe they are reduced in the higher degree by general mechanical oral hygiene as practiced today. While the tooth pastes and powders as used with brushes have no preventive or curative powers in regard to gingivitis they do help in a general clean up and in this way reduce the severity of gingival infections.

The table shows 2 082 cases or 53.1 per cent with outstanding mucelial threads or leptothrix accumulations. We are not in a positive position on this organism and its relationship will be discussed later. The yeasts and fungi are not listed but their incidence of occurrence is very low and they have no direct etiologic bearing.

The few protozoa other than endameba were noted and cited for appropriate treatment but they play practically no part as general etiologic factors in gingivitis. Where present in a given case their activity may irritate and open up portals of entry, and in some cases their persistence is tenacious in

TABLE II
GRADATIONS OF POSITIVES IN NUMBERS PER 20 FIELDS OF EACH EXAMINED

	NUMBER	PER CENT
Spironema 1 plus	1,205	31.6
Spironema 2 plus	849	23.2
Spironema 3 plus	1,212	31.7
Spironema 4 plus	537	13.4
Endameba 1 plus	1,862	65.0
Endameba 2 plus	574	20.1
Endameba 3 plus	322	11.3
Endameba 4 plus	90	3.1

spite of treatment Hinshaw¹⁴ reported three cases *Trichomonas buccalis* out of 64 cases cultured or 4.6 per cent. This is considerably higher than our own incidence but not enough to influence the general conclusions in any way.

One hundred per cent showed the presence of pus (that is exudative white blood cells dead or alive in sufficient numbers to be called pus). This represents another point in the inflammatory nature of every case of gingivitis. At the same time, of the 428 cases called normal, 145 or 33.8 per cent had some pus while 283 or 66.2 per cent showed absolutely nothing.

As has already been stated the smear reports in this paper are based mainly on fresh smears examined as recorded. The stained smears were not noted separately, indeed they are no longer carried out routinely and are now used only to bring out certain special features as already described. It is advisable to check the fresh smears with stained preparations until thorough familiarity with the fresh is obtained.

In the examination we are in the habit of first determining the presence of endameba by a search of at least twenty fields and noting the number per field. They are readily found and have many definite characteristics. The important feature of the fresh smear is the movement of endameba which makes them definite. We next determine the presence of spironema noting number, shape, size, and motility, next the relative number and clumping of bacteria and pus cells and any predominating bacteria. In the latter search, fusiform bacilli and leptothrix are included. Finally we are on the lookout for yeasts (we think that routine stained specimens would show a higher incidence of occurrence but at least for the time being they are relatively unimportant) protozoa other than endameba and anything unusual. This takes in all about fifty fields in the study.

Cultures from gingival sulci show a wide variety of organisms both aerobes and anaerobes. While this variation is marked in a large number of cases, the flora of a certain mouth is relatively constant when it is kept under cultural control from day to day or month to month. Unlike some lesions of the nose and mouth in which a single organism either develops at the expense of others or in its development becomes repellent to others and the cultures are characteristically single organisms the gingival sulci as a rule give a very mixed flora. Three, four or more different species is the rule. The coccil group predominates and anaerobes are always present. In the complete study of a given case where clinical signs continue in spite of treatment, where control cultures are advisable before going into deeper structures, or where

a flora is desired for the preparation of a vaccine material from gingival pockets should be planted on several different types of media and careful fishing and pure culture isolation carried out. This paper is now too long and cannot include details of cultural studies, but suffice it to say that the gingival bacterial flora as a group entity plays an etiologic role in gingivitis and a much more important one in the secondary deeper tissue invasion with the development of focal areas of infection.

The sections of gingiva so far studied are quite uniform in their appearance. We are not including here the more complex pathology of the different types of so called periapical cysts and periapical pathology. We have a number of jaws removed at autopsy including gingivae, teeth and bone which will be the subject of a separate report. The normal gingiva shows very little other than the epithelial covering the loose connective tissue and lymph spaces with thin walled blood vessels and a few submucous racemose glands. The latter are not numerous and are rather rudimentary in type. When the gingivae are the seat of inflammatory changes round celled infiltration of the wandering lymphocytic and plasma cell forms predominate. The polymorphonuclears are scattered and occasionally definite small abscesses are to be seen. Where the process is advanced surface ulceration is present with its line of necrosis, and often against this mailed infiltrating leucocytic lines of demarcation are made out. As the process advances and in the more chronic cases of long duration, much irregular fibrogenetic tissue with strands of old hyalinized bands are present. In the midst of this tissue microorganisms are readily demonstrable by appropriate staining as has been shown by Beckwith, et al.¹⁸ By careful technique these organisms can be cultured, and the plates rarely show more than two types and more often a single organism is obtained. This will always bear a direct relationship to the same organism which can be cultured from the gingival sulci of the same case before its surgical removal. Some form of coccus is the predominating type.

Twelve cases were studied every day for thirty days with a daily comparison of results. Eight of these cases were gingivitis of various stages and four were normal controls. They were studied clinically and by smear and culture. They were submitted to recorded control treatments and minute detail as to any influencing factors were recorded. The persistent and tenacious qualities of the various infecting factors were striking but just as striking was the improvement both clinically and bacteriologically under treatment. The limitations however of our present knowledge of efficient remedies was quite apparent from this study. The periodicity of infection was partially established, and at least a tentative program was mapped out for the necessity and times of continued treatments. From the cultures of this group it was found that all sizes, shapes and activities of spirillum-like organisms could be obtained, and we now believe that they all belong to the one group *spirochaeta*. Subject to further proof it is our opinion that the so called fusiform bacilli are really mucous threads segmented from a parent leptothrix body which are both gram positive and gram negative according to their stages which are both motile and nonmotile also according to their stages and which in the motile form angulate and become spirals during a

cycle of their development. It is very difficult to grow these organisms, at least in our hands, but from a composite study of a large number of cultures, again subject to proof, we believe the spiral organisms present in such large numbers in so many mouths are stages, through motile and nonmotile bacilli which are now known as *B. fusiformis* and are now generally considered in symbiotic relationship, of a parent fixed mycelial or leptothrix grouping of the so-called higher forms of bacteria.

The changes in H-ion concentration of the exudates of gingival sulci as compared with saliva were carried out by the micro method, using the LaMotte colorimetric set-up. In the group the twelve cases were included and 256 observations were made. This number is entirely too small to draw definite conclusions from, but the figures are rather striking. The gingival exudates are quite acid and vary somewhat under different conditions, probably due to the cultural influences of acid-forming bacteria. The exudates are decidedly more acid than mouth salivas. The average P_H of a gingival sulcus, the seat of gingivitis, is 5.72, with a range of 5.4 to 5.8, the lowest 4.6 and the highest 6.1.

THE RELATION OF THE BACTERIOLOGIC FLORA TO GINGIVITIS

The bacteriologic flora of gingival sulci in relation to periapical cultures is, of course, complex but is apparently quite constant and persistent as far as each mouth is concerned. That is, there is not much change in types of the organisms from day to day. The predominating cocci, *Staphylococcus hemolyticus* and nonhemolytic, aerobic and facultative anaerobic, gas-producing and acid-forming, *Streptococcus pyogenes*, hemolytic and nonhemolytic and the large group of *B. hoffmanni* are quite constantly present in all mouths. The relationship of this flora or at least of one or two characteristic organisms, to subgingival areas, deep periapical cysts, root canals and infectious osseous cystic mandibular osteitis and sinus involvement are striking, and in a given case the same organisms can be isolated from the gingival as well as from the deeper sources. Of course, there come to one's mind immediately questions of contamination. Let me say here, when one first starts to work on this problem bacteriologically technical difficulties beset one at every hand, but with experience and a complete knowledge of working conditions contaminations can be completely and entirely ruled out and one's results can at least keep one's own mind at rest. The secret of success is scrupulous attention to technical detail and plenty of checking. The only other factor is experience. It was from these bacteriologic findings and checks that we firmly established, at least in our own minds, the fact that gingivitis is so often a multiple form of infection and of much greater importance than root canals in establishing deeper and more potent foci. When this fact was realized and applied to clinical observation and treatment, the results obtained improved markedly and have been most gratifying and confirmatory.

The bacteriologic flora in its relationship to the gingival pathology is worth considerable thought, and upon this relationship hinges the relative importance of each of the organisms enumerated and the parts they play. Assuming the gingival sulci contain a varied flora, is it fair to assume that any

one organism is more important than any other. What proofs are we able to set forth as to the importance of each? In the first place the cultural characteristics of all organisms found must be fully known before any animal experimentation can be successfully made and Koch's postulates fulfilled. It is said that spirouema for instance, grows anaerobically but to accomplish this is extremely difficult. The endamebae have been grown, but to get rich cultures easily is almost impossible at the present state of our knowledge. Until these two organisms are more thoroughly mastered we feel that any animal work would be incomplete. In order then to give a relative importance to these different bacteria, we must turn first, to the character of the pathology, and second, to the results of chemotherapeutic attacks directed at each with a careful notation of good and bad changes obtained.

The pathology suggests general chronic infection, that is, small doses constantly received with now and then a sharp flareup with suppuration. Endameba has never been found in the gingival tissue. We have looked for it often and still feel that at times it must actually invade the tissue although this is not its habit. Spirouema has never been successfully demonstrated in the tissue, but more painstaking search must be made for this. The other groups of organisms have been demonstrated both by section and by culture in the tissue, and the tissue shows pathologic evidence of reaction against these organisms. Endameba spirouema and organisms are found more or less constantly in gingival sulci showing evidence of gingivitis. It is therefore fair, and we believe reasonable to attach the proper etiologic significance to these organisms in so far as our present state of knowledge will allow.

The spirouema under most conditions in the mouth are extremely active. They are actually belligerent against cells, they have a rapid mechanical cork screw movement which is directed actually in mass attack so that they are able mechanically and violently to shile a pus cell. Multiply this action by the billions which are present in gingival sulci and mechanically then they are the finest kind of a stimulus to set up traumatic reaction. This is entirely aside from any toxic action which they may possess against cells.

The endameba is much slower in its action but just as powerful. Mechanically it is able to wedge itself in and between cells and debris and reminds one of the relative strength of an elephant in ordinary underbrush. On the other hand it is quite likely to have an enzymic digestive toxin, and its powers of phagocytosis are well known. There is doubt in the minds of some but they need be troubled no longer if they will but observe that an endameba will engulf a red blood cell. We have seen this on numerous occasions. It was first seen by us in a preparation of Dr. Allen J. Smith's and since on several occasions we have seen this repeated. The red blood cell is attracted to the surface of the endameba as though by a potential surface tension. It is not actually pulled in by the pseudopods but seems to melt into the body of the endameba which is always in strong activity when this is accomplished. It is a matter of many minutes, as many as twenty, before the red blood cell is actually within the body of the endameba but once inside its identity is rapidly lost. It seems to be quickly consumed as though by solution. We have spent many hours watching the movements of

endameba, and while one in activity does many queer things, all movements except the digesting of bacteria and red blood cells seem to be absolutely purposeless. This protozoa is composed of a very delicate chemical mechanism, and the least change of the surrounding medium throws it into dissolution. The endamebae then not actually in the tissue but in the gingival sulci are strong mechanical factors and traumatic stimuli for inflammatory reactions.

With the powerful initiating stimulus of both endameba and spirochete setting up and continuing an inflammatory reaction, the smaller but more powerful bacteria, many of which are not only motile but dart across the field so fast that they cannot be followed, use the gingival sulci as a portal of entry to the subgingival, periodontal, periosteal and osteal tissue and continue a more violent and in a way a more important reaction. This leads to tissue pathology, extensive gingival inflammation, multiple foci of infection and from these organisms are picked up by the lymphatics and the blood and carried to distant and varied parts of the body. This is a slow process, an intermittent process, and mostly, for which we can be thankful, in very small doses. Such bacteria, lodging in distant parts of the body, set up reactions characteristic to their type, influenced by their selective activities, and there is seen the varied symptomatology of what we have spoken of as distant manifestations of oral dental foci of infection.

One of the other factors may be present in a mouth, they help to prepare the field and continue as an irritant, in order that the bacteria may do their actual fighting work. When it is remembered that all the bacteria want is a place to live and some food, and when they find such excellent conditions in gingival sulci for cultural elegance, is it any wonder that gingivitis is so prevalent. These other factors, what Vastine speaks of as the negative phases of the subject, are lack of dental oral hygiene, uselessness of dental creams and powders from a chemotherapeutic standpoint, inefficiency of the toothbrush method in reaching gingival sulci, the mechanics of crowns, bridges, plates and dentures, cavities and dental caries, calcification of gingival exudates, traumatism of chewing, accumulation of foodstuffs in and between teeth, sugars, proteins, and the intravital relation of systemic diseases. These and maybe others play a part in allowing gingivitis to start or in helping to continue it and in that sense are contributory factors, but in no sense are they to be considered prime etiologic factors. They are most important, however, in accomplishing a final cleanup when it comes to treatment.

PRINCIPLES OF TREATMENT IN GINGIVITIS

This paper is now entirely too long to include treatment, but the principles as worked out by Vastine in complete cooperation with the laboratory must be at least discussed. In the first place the bacterial flora of the gingivae with gradations of positives must be studied and used with frequent checkup for all subsequent treatment. It is perfectly proper for the laboratory to suggest types and dosage of chemotherapeutic measures. *The successful treatment does not depend upon any one or two procedures, methods, or drugs but a mass attack using every available method against conditions found in*

the individual mouth There is no one specific cause for gingivitis and there is no one specific treatment After all possible data as to etiologic factors have been obtained in a given case, appropriate methods of correction are then to be carried out If caries and caries are present, they must be corrected If bridges, crowns, etc., are wrong they must be corrected (we have seen many beautiful mechanical arrangements which it was absolutely necessary to sacrifice before a given case could be cleaned up) This is a matter of much education, but it might as well be realized sooner than later If there are any gross collections of so called tartar or calculus which are traumatic stimuli, they are to be removed but the general process of scaling, with injury to soft tissues and especially to the tooth is absolutely condemned and the general practice of scaling might as well stop right now The same thing holds good with all the various manual polishers, stones, and wheels which do more damage than good Every injury to soft tissue and the tooth must be repaired The tooth has little or no regenerating power, injury to it leads to caries and injury to the soft tissues leads to or increases the chances of portal of entry for microorganisms The dentists as a whole are of course, antagonistic to these statements, but that is due to the teaching in our dental schools today, and to the striking lack of knowledge of fundamental pathology The crying need is a general cooperation in the entire study of this group We have tried to show the relationship between gingivitis and deeper focal areas and, of course, the cleanup of both areas must go hand in hand Any other pathologic changes in the nose and mouth such as tonsils, or sinuses which are just as important, must have an absolutely cooperative cleanup When all contributory factors have been recognized and means taken to control them, local chemotherapeutic measures started at the same time or before some parts of the cleanup, as for example before dental foci are touched, should be vigorously carried out Some patients may require treatments once a week some once a day, some every two hours all depending upon the clinical characteristics and the violence of the infecting organisms Some may clear up promptly, and some may require treatment for months After a general cleanup a return check up should be carried on about once a month The most severe cases after they are under control may be kept so by a treatment varying from once every two to three weeks to two to three months So far as our chemotherapeutic knowledge goes today, there is nothing which will clean these cases up and keep them clean forever They all tend to recur, and the very best that can be hoped for is control This however, fortunately can be carried on with almost one hundred per cent of assurance if—and so far as we know only if—all the conditions enumerated are established and maintained

The therapeutic measures are many, but the aniline dyes asphenamine emetine, Dakins' solution hot water irrigations, sodium perborate, hydrogen peroxide, combined in some combination to fit the bacteriologic flora are the best remedies we have found so far Mouth washes are valuable merely as cleansers and any tooth powder or paste is good for cleansing reasons none of them have any influence directly on the specific factors of gingivitis

The method for administering various drugs is most important, and that

advocated by Vastine is the most direct and efficient so far devised. The use of the rubber cup gets the drug in the dilution which is best suited directly to the bacteria in the gingival sulci without injury to the tissues. Swabbing, needles, instruments, syringes, pressure devices have all been tested out, and none of these have the efficiency of the small rubber cup. The length of time, the concentration, and the nonirritant chemical character of the drug are all very important as is the interval between treatments.

We are opposed to all operative procedures on the gums, that is in the treatment of gingivitis per se, except in special cases, for example, where one pocket is almost a sinus and a small incision would facilitate drainage or the application of the treatment.

SUMMARY AND CONCLUSIONS

1 The problem of gingivitis is one of the largest and most important phases of medicine as it stands today. It is far-reaching in that over 90 per cent of our population have it in some form or another. Its controversial stage involves mainly the dentists while the medical men are realizing its importance and demanding a more careful attention to it.

2 The term "pyorrhea," representing as it does the end-stage of a disease, should be immediately discarded for a more inclusive term. Gingivitis qualified by acute, subacute, or chronic is suggested, since the disease is in all stages an inflammation of the gingivae.

3 The disease in the majority of cases goes unrecognized in the early stages, runs a long-continued course, finally terminating in pockets, destructions, recessions, and loss of teeth. It is one and the same thing in different degrees of severity and stages of advancement.

4 A plea for early recognition and adequate prophylaxis is made, and if carried out, many of the terminal changes will be avoided.

5 The gingival portion of an acute trench mouth is one and the same thing as other gingivitis and the acute flareup is due to cultural characteristics and conditions. The acute ulcerative and exudative gingivitis progresses to stomatitis, the condition now recognized as trench mouth.

6 The study and future treatment of gingivitis depends upon an intelligent and close cooperation between the laboratory and the dentist. When this exists with the patient's cooperation, results may be expected in every case. This has a good many "ifs," but the complexity of the problem demands it at the present development of our knowledge.

7 The high points of a study of over 4,000 cases are reported from an etiologic standpoint, and the following conclusions are drawn:

- A 96.4 per cent show the presence of spirillum
- 71.6 per cent show the presence of E. gingivalis (Gros)
- 66.6 per cent show a definite bacterial flora

B These three groups of organisms alone or in combination are the direct etiologic factors in every case of gingivitis, and all other factors present are contributory and subservient.

C The spirochete and E gingivitis and bacteria act mechanically, finding an ideal culture bed in the gingival sulci in which to set up a gingivitis. The toxic factor of each is probably secondary but equally important.

D The spirochete and E gingivitis reside probably only in the gingival sulci while the bacteria penetrate the gingivae, lodging in the deeper structures where they act as potent foci of infection.

E There is evidence that the so called symbiotic relationship of Vincent's spirillum and B fusiformis does not hold but in reality these are cycles or phases of a higher form of leptothrix.

F It is suggested that the term Vincent's spirillum is misleading and should be discarded as well as the entity Vincent's angina.

G Until further light is thrown upon the spirillum-like organisms of the mouth, we believe that all of these organisms are one and the same genus and fall best according to the manual of *Determinative Bacteriology* under order VI spirochetes. Family 1 spirochaetaceae Genus IV spirochete,* 12 *Spirochete vincentii* (Blanchard). For the time being we think that any association, as a symbiosis or as a necessity for diagnosis, with any fusiform bacilli may be disregarded. We further believe, under Genus V treponema of the same family spirochaetaceae that 3 *Treponema microdentium* (Noguchi), 4 *Treponema macrodentium* (Noguchi), and 5 *Treponema mucosum* (Noguchi) are one and the same as spirochete with cultural variations.

H The disease known as trench mouth is nothing more than a flare up having its origin in a gingivitis and as such should be spoken of as acute ulcerative gingivitis and stomatitis due to spirochete as a part of the general problem of gingivitis.

I A method of study from the laboratory standpoint is offered with a standardization of reports.

J Gingivitis is not due to a single specific factor but to a combination of infections which must be studied in every mouth and evaluated for that individual.

K We believe that the evidence bacteriologically, pathologically, and clinically is sufficient to show definitely the relationship between the organisms encountered and the clinical reactions and to state that all forms of gingivitis have a common etiology with degrees of reaction.

L We believe that the vast majority of cases start insidiously in young life and continue slowly but progressively terminating surely and eventually in marked and destructive changes in the middle and later decades.

8 From an intensive study of twelve cases with daily examinations, the constant character of the gingival bacterial flora was striking.

9 The H ion concentration of gingival exudates would seem to be influenced

Where the generic name spirochete appears in this paper it should be substituted by Borrelia. The generic name spirochete used in the first edition of the *Manual of Determinative Bacteriology* is invalid because of its prior use for other organisms and in the second edition it is changed to Borrelia.

by the gas-forming acid organisms of the flora and to maintain in part an acid reaction, which is greater than the saliva of the mouth

10 The gingivae, the seat of gingivitis, offer the largest and most important single portal of entry for microorganisms and as such are the most potential sources of foci of infection in the body

11 Organisms picked up by the circulation from the gingival foci are responsible for many distant manifestations and metastatic foci

12 This latter statement makes a direct connection between this source of infection and such diseases as arthritis, rheumatism, endocarditis, myocarditis, nephritis, anemia, and several more

13 The treatment depends upon a careful consideration of all contributory factors present in a given case and their complete correction. With this, local chemotherapeutic administration in proper amounts, concentration, time, method, and control carryon, the problem of gingivitis difficult and complex may be solved

14 From all these different angles it is finally concluded that gingivitis is an entity only in so far as it involves the gums, it is due to several factors, primarily infection with spirochete, *E. gingivalis*, and the bacterial group playing the important rôle, and all other conditions are secondary and contributory. Gingivitis is a chronic disease of long duration in the vast majority of cases, starting mildly but ending in necrosis and destruction. Gingivitis is subject to acute flarups as a part of the general disease. Gingivitis will subside and can be absolutely kept under control by appropriate therapeutic measures intelligently applied

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LABORATORY METHODS

THE CULTIVATION OF TUBERCLE BACILLI AN IMPROVED METHOD FOR ISOLATION FROM TUBERCULOUS MATERIALS*

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SINCE the introduction in 1915 of Petroff's¹ method for the primary isolation of tubercle bacilli from tuberculous materials, there have been few advances or improvements in methods for isolating tubercle bacilli, and this method has been considered the best and most efficient by the majority of laboratory workers as well as by clinical pathologists. More recently Lowenstein suggested the use of acids as reagents for treating the tuberculous material before planting on mediums for isolating the tubercle bacilli. The advantages or disadvantages of the acid methods over the sodium hydroxide method have not been thoroughly investigated. The clinical pathologist who wishes to isolate tubercle bacilli from tuberculous materials either for diagnostic or experimental purposes would prefer a method yielding pure cultures in as short a time as possible and with the least expenditure of labor and time for performing the manipulations required. The timesaving factor plays an important role in the selection of methods of diagnosis especially and particularly for the isolation of tubercle bacilli, since these organisms grow only slowly at best. For this purpose and as a part of the program of the Research Committee of the American Society of Clinical Pathologists a critical study was made of the relative efficiency of the alkali (Petroff's) and acid (Lowenstein's) methods as well as of several other methods considering especially the efficiency of the reagents and the suitability of the mediums.

A study of the methods for the isolation of tubercle bacilli from tuberculous materials can conveniently be divided into two parts namely one dealing with the efficiency of the reagents in killing off contaminating organisms and the other the efficiency of the medium for the growth of tubercle bacilli. Although these studies were concerned primarily with isolation from positive sputums, the results apply equally well to other tuberculous materials, such as urine, feces, and tuberculous tissues.

I AN INVESTIGATION OF THE REAGENT USED FOR EXCLUDING CONTAMINATING ORGANISMS

Among the various reagents used in isolating tubercle bacilli antiformin² (Brown and Smith Paterson, Griffith), formalin (Spengler), and sodium hydroxide (Petroff³) have played prominent parts, but the latter was found from our previous studies to be far superior to the other two methods.⁴ Luke wise glycerine has been suggested as a means of destroying the contaminants

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but this was also found to be of no service in our laboratory⁶ More recently Dold⁷ suggested the use of urea as an efficient agent for destroying contaminants, and Lowenstein⁸ advocated the use of sulphuric, hydrochloric and other acids for the same purpose

In studying germicides for destroying the contaminating organisms, two factors must be given consideration First, the efficiency of the reagent as a germicide for the contaminants, and second, the toxicity of this reagent for the tubercle bacillus, the organism to be isolated The first of these factors can be satisfactorily studied by mixing sputum with a definite quantity of the reagent and seeding the final product of such treatment on a standard medium of known efficiency, such as Petroff's gentian violet egg medium, and then determining the number of contaminations resulting in the seeded culture tubes after incubation The second factor can be determined by taking a definite weighed quantity of tubercle bacilli and, after making a fine suspension in normal saline solution, mixing a definite portion with a definite volume of the reagent and then seeding the final washed or diluted product on to a standard medium and noting the growth of the tubercle bacilli By the use of these methods we studied the relative value of different reagents for the isolation of tubercle bacilli and have concluded from these studies

1 *Urea and Sodium Carbonate*—Urea possesses an excellent solvent action while sodium carbonate exerts a precipitating effect upon the mucus-like materials in sputum and tissues By means of these reagents tubercle bacilli can be isolated only in a few cases, because they permit frequent contamination of the culture mediums, even when the sputum or other tuberculous material is treated with a high concentration of the reagents Urea and sodium carbonate are, therefore, unsuited as reagents for destroying the contaminants for the purpose of isolating tubercle bacilli from contaminated sources

2 *Ammonium Hydroxide* is an efficient germicide and permits the growth of only a small percentage of contaminants even when sputum is treated with a low concentration of the reagent This reagent, cannot, however, be employed for isolating tubercle bacilli as these bacilli are also very susceptible to its toxic action, and the same low concentrations that permit the growth of contaminants are also injurious to tubercle bacilli

3 *Ammonium Carbonate* is an inefficient germicide for contaminating organisms and yields too frequent contamination even when sputums are treated with a high concentration of the reagent At the same time this reagent is too toxic for tubercle bacilli, and is entirely unsuited for the isolation of tubercle bacilli from contaminated sources

4 *Sodium Hydroxide*, the reagent recommended by Petroff is an efficient germicide to contaminating organisms and is also of low toxicity to tubercle bacilli Our results using Petroff's medium gave an efficiency of about 55 per cent isolation, using all growths even of small amount as a criterion, and about 16 per cent contamination with positive microscopic sputums, seeding about 3 tubes from each sputum These figures compare favorably with most previous investigators

5 *Sulphuric Acid and Hydrochloric Acid*—The acid reagents recommended by Lowenstein for isolating tubercle bacilli are efficient germicides toward contaminating organisms and possess little toxicity toward tubercle bacilli in suitable concentrations. They also possess the advantage of permitting an earlier appearance of colonies as compared with cultures when sodium hydroxide has been used. In an experiment with four positive sputums, using the sulphuric acid reagent and on the same sputums the sodium hydroxide method, all seeded on the same medium, growth occurred several weeks earlier with the sulphuric acid reagent than it did after the use of sodium hydroxide. The percentage of positive isolations were also greater with sulphuric or hydrochloric acid (about 65 per cent and 10 per cent contaminations) when the proper culture medium was used. The superiority of sulphuric and hydrochloric acid over sodium hydroxide is probably accounted for by the fact that in the sodium hydroxide method, the heavy slimy fluid produced by mixture of sodium hydroxide and sputum prevents efficient sedimentation of the tubercle bacilli during centrifugation, while with sulphuric or hydrochloric acid, the sputum reagent mixture produces a flocculent precipitate upon dilution which materially assists in sedimentation of the bacilli during centrifugation. Sulphuric acid proved slightly superior to hydrochloric acid because the former has a wider range of useful concentration than the latter.

In summarizing the part of the investigation concerning the value of the reagents tested, it is concluded that urea and sodium carbonate are unsatisfactory reagents for the isolation of tubercle bacilli because frequent contamination occurs after their use, they being inefficient germicides for contaminating organisms in a concentration and time interval tolerated by tubercle bacilli without appreciable injury. Ammonium hydroxide proved too toxic toward tubercle bacilli in concentration and time interval suitable for destroying the contaminants. Ammonium carbonate possesses the same disadvantages. Sulphuric acid, hydrochloric acid, and sodium hydroxide are all suitable in that they destroy contaminants within a time interval of exposure and concentration in which they are innocuous to tubercle bacilli in tuberculous materials. Of these three reagents an equal volume of 6 per cent sulphuric acid and thirty minutes incubation (37°C) proved superior with 3 per cent hydrochloric acid in equal volume for from fifteen to thirty minutes incubation (37°C) next in value, and an equal volume of 2 per cent sodium hydroxide for from fifteen to thirty minutes least efficient in yielding positive cultures of tubercle bacilli as well as in destroying contaminants.

II THE VALUE OF DIFFERENT CULTURE MEDIUMS

To prevent further the growth of such contaminating organisms as were not destroyed by the sodium hydroxide reagent Petroff incorporated gentian violet as a bacteriostatic agent in Dorset's egg medium. The gentian violet possesses an added advantage of making the detection of colonies of tubercle bacilli on the medium easier. Prior to the World War of 1914-1918 G. Grubler and Company, of Germany, was the sole distributor of dyes in this country. After the war the dye manufacturers and biologists of the United States organized a "Commission on the Standardization of Biologic Stains"

in order to stabilize and standardize the dyes used in America. Accordingly on the recommendation of the commission, dye manufacturers are now placing on the market "crystal violet" or "methyl violet" as a substitute for Grubler's gentian violet.

A number of samples of the dyes manufactured by the leading dye manufacturers in this country and kindly furnished to us by Dr. H. J. Conn, Chairman of the Commission on Standardization of Biologic Stains, have been tested for their action on tubercle bacilli. The samples of the dye tested included the following: (1) crystal violet from the National Aniline and Chemical Company, sold as gentian violet, (2) crystal violet from the Coleman and Bell Company, sold as gentian violet, (3) crystal violet from the Empire Biochemical Company, sold as gentian violet, (4) crystal violet from the Hartman-Leddon Company, sold as gentian violet, (5) methyl violet from the Coleman and Bell Company also sold by them as gentian violet and (6) crystal violet from the Coleman and Bell Company, sold as crystal violet. The study of the relative value of these dyes indicates that when they are present in high concentrations (0.01 per cent or over) in mediums they inhibit the

Type of Medium	Amount of Seeding														
	Milligram					Thousandths Milligram			Millionths Milligram			Billionths Milligram			
	25	10	1	0.1	0.01	1	0.1	0.01	1	0.1	0.01	1	0.1	0.01	
Long's															
Glyc Agar			Questionable									No Growth			
Petroff's															
Dorset's			Good Growth												
Calmette's															

Fig. 1.—Growth chart for a strain of virulent human tubercle bacilli (Gluckson) when seeded in dilute suspension on different media.

growth of tubercle bacilli, while in lower concentrations they exert no demonstrable injurious effect. Any of these dyes can be used in egg mediums in the same concentration (1/10,000) as originally recommended by Petroff for Grubler's gentian violet.

In a previous study on the relation of food accessory substances (vitamins) to bacterial growth⁹ a suspension method for seeding tubercle bacilli in graded dilutions was used in order to compare the efficiency of different mediums for the growth of tubercle bacilli. This same method was used in the following investigation for the purpose of comparing the efficiency of different mediums for the growth of tubercle bacilli. A number of interesting findings have resulted from this study. Fig. 1 depicts at a glance the relative efficiency of the different mediums tried when tubercle bacilli are seeded on them in graded amounts from dilute suspensions. It is evident from the figure that growth of virulent human tubercle bacilli on the nonprotein medium of Long occurs only when the medium is seeded with suspensions containing 25 mg. or more of bacilli per cubic centimeter. When the concentration of the suspension is from 0.1 mg. to 10 mg. per cubic centimeter, there may or may not result growth, while when the inoculum contains only 0.01

mg or less of bacilli per cubic centimeter, no growth occurs in any of the tubes seeded. On 5 per cent glycerin nutrient agar medium, growth takes place when the concentration is 10 mg or more of bacilli per cubic centimeter, while a concentration of from 0.01 mg to 1 mg of bacilli per cubic centimeter gives uncertain growth. With 0.001 mg or less bacilli per cubic centimeter, seeding fails to give colonies on the glycerin agar medium. On Petroff's gentian violet egg medium, growth occurs when the suspension contains 0.1 mg or more of bacilli per cubic centimeter while between 0.001 and 0.01 mg per cc uncertain growth results and with 0.0001 mg or less per cubic centimeter the seedings entirely fail to grow. With Dorset's and Calmette's medium, these points of extinction have not yet been fully determined, but it seems that with Dorset's medium growth always takes place at the concentration of 0.000 001 mg or more while below 0.000 000 01 mg growth does not take place. On Calmette's medium growth occurs down to 0.000,000,001 milligram with certainty. From this experiment it is also to be noted that on Calmette's potato cylinder medium, tubercle bacilli grow more luxuriantly and develop visible colonies earlier than on the other mediums tested. This was especially so with the high dilutions of the bacilli. In an earlier study¹⁰ it was computed that 1 milligram of tubercle bacilli taken from a culture contained between 5.01 and 7.56 billion tubercle bacilli or theoretically speaking, if grinding was thorough, a suspension of 0.000 000 000 1 mg of bacilli per cc should contain only a few bacilli. Since only 3 or 4 drops (about 0.3 cc) of suspension were used for seeding the culture tubes this dilution would give only one or two colonies or none at all. Although definite statements are not justified from our preliminary experiments, it is interesting that in this concentration a few colonies of tubercle bacilli developed on the potato medium. The guinea pig inoculation method for determining the presence of tubercle bacilli is considered the most sensitive available for this purpose at present and has proved to be one of the most important means for the diagnosis of doubtful cases of tuberculosis. The use of the guinea pig for this purpose has a number of disadvantages in not being easily accessible being more expensive, and in requiring greater facilities than the culture method. If it were possible to use the culture method with equal efficiency to the animal test, it would make possible not only a test susceptible of repeated examinations but would also prove to be time and labor saving. This is now being investigated in our laboratory.

Although the potato cylinder medium proved to be the most satisfactory medium for the growth of tubercle bacilli its use unmodified for the primary isolation of tubercle bacilli was impaired by the fact that contaminants also thrived on it. The contaminations diminished the percentage of isolation of tubercle bacilli from sputum as compared with the results on Petroff's gentian violet egg medium. Lowenstein attempted to use mercuric chloride in the potato medium in order to prevent the growth of contaminating organisms but we have found this reagent highly toxic to tubercle bacilli as well as to other organisms. To remedy this defect of the potato cylinder medium crystal violet was introduced thus we have succeeded in preparing a medium apparently exceeding the value of the original potato cylinder medium in pro-

ducing an acceleration of the growth of the tubercle bacilli. This stimulating effect of the small amount of crystal violet in the medium upon the growth of the tubercle bacilli may be analogous to the stimulation in the growth of plants occasioned by the presence in their nutrient mediums of a minute amount of the salts of heavy metals, which if present in a larger concentration exert a deleterious effect. In an earlier report it was also pointed out that a small amount of carbon dioxide in the surrounding atmosphere accelerated the growth of tubercle bacilli while higher concentrations exerted a harmful influence. Thus, it is conceivable that crystal violet in low concentration accelerates the growth of tubercle bacilli, while in higher concentrations it may retard their growth.

The concentration of crystal violet still suitable for use in the potato cylinder medium for the isolation of tubercle bacilli is 0.01 per cent or less. The

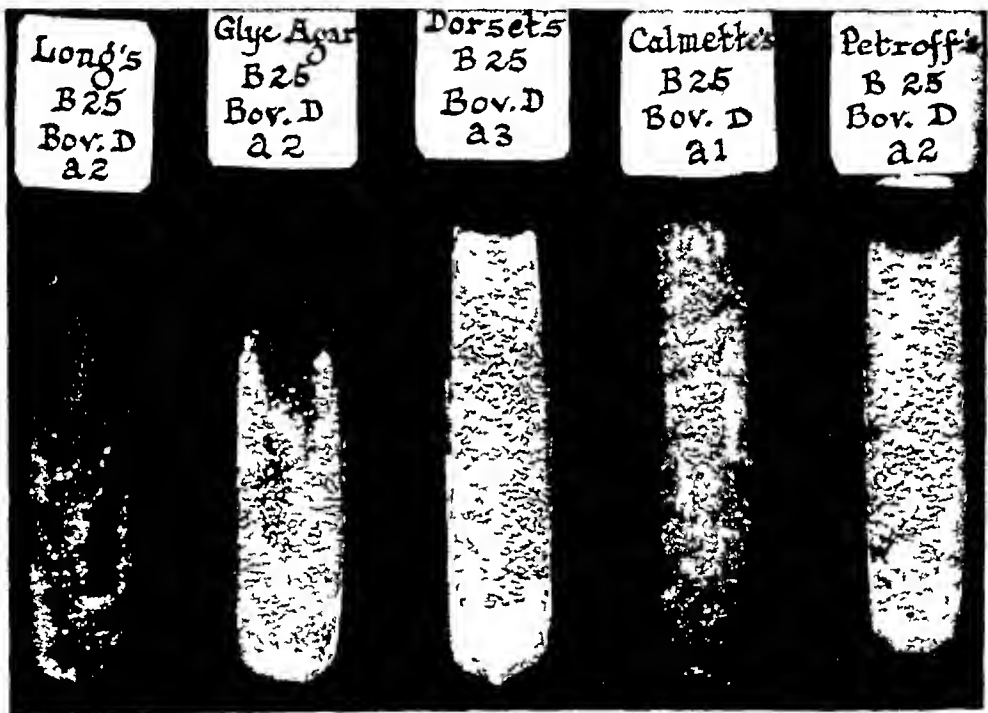


Fig. 2.—The growth of tubercle bacilli (bovine) on Long's nonprotein medium five per cent glycerol agar, Dorset's plain egg medium, potato cylinder nutrient broth, and Petroff's gentian violet egg medium 5 weeks after seeding with a few drops of suspension containing 25 milligrams in a cubic centimeter and incubating at 37° C. Human bacilli grow about like bovine on these mediums. Note less profuse growth on Long's and potato medium with heavy seeding.

most favorable concentration in our experiments was found to be 0.003 per cent or about a 1:75,000 dilution. The crystal violet potato cylinder medium as well as the plain potato cylinder medium proved superior to Petroff's and other mediums tested, in so far as the first appearance of colony formation was concerned after seeding with tubercle bacilli. This combined with the use of the 6 per cent sulphuric acid reagent resulted in a saving of several weeks time in many instances of isolation of tubercle bacilli from contaminated sources.

Regarding the substances present in the potato medium favorable to the growth of tubercle bacilli little is known and in elucidation of this it may be noted that Nocard and Roux's glycerin nutrient agar medium contains sufficient glycerin and bouillon for the growth of tubercle bacilli, yet it fails to support growth consistently when the number of bacilli are less than 10 milligrams per cubic centimeter. Lowenstein found that potato cubes dipped in saline solution alone may be used for the cultivation of tubercle bacilli. From all the evidence available it would appear that the stimulating substance for the growth of the bacilli is present in the potato rather than in the bouillon or glycerin. The studies of Thjotta and Avery¹³ have shown that there is present in potato two distinct and separable substances which accel-

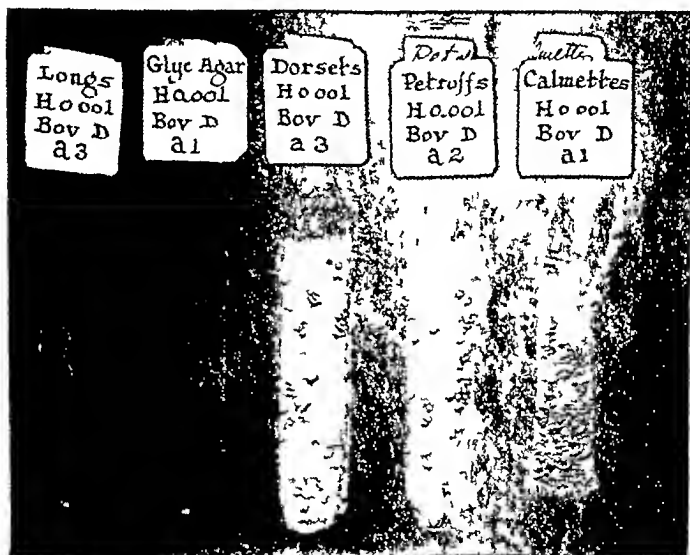


Fig. 3—The growth of tubercle bacilli (bovine) on Long's glycerol agar, Dorsets, Petroff's and potato medium 6 weeks after seeding with a suspension containing 0.001 milligram in a cubic centimeter and incubating at 37°C. Note absence of growth on Long's and glycerol agar mediums with this seedling and more profuse growth on the potato medium than on Dorsets medium and least growth on Petroff's medium.

erate the growth of hemophilic bacteria, one a heat stable so called X substance and another a heat labile vitamin like substance. In a recent work¹ it was found that tubercle bacilli thinly seeded in small amount on synthetic nonprotein medium fail to grow while in the presence of substances rich in vitamin B there is a marked acceleration of growth. This might incline one to believe that the active principle in potato suitable for the growth of tubercle bacilli may possibly be a vitamin B like substance alone or vitamin B like as well as the X substance of Thjotta and Avery. Potato starch was

tested as a substitute for raw potato, but it was found that although the potato starch promotes the growth of the bacilli when the medium is heavily seeded, with small amounts of seeding no stimulating effect was discernible. The growth-promoting principle appears, therefore, to reside mainly in the raw potato and this is being submitted to further study.

AN IMPROVED METHOD FOR THE PRIMARY ISOLATION OF TUBERCLE BACILLI

As a result of a study of various reagents and mediums the following method is proposed as being an advance over methods previously used for this



Fig. 4—The growth of tubercle bacilli (virulent human) on Petroff's, Dorset's and potato mediums 5 weeks after seeding with a suspension containing 0.000,000,001 milligram in a cubic centimeter and incubating at 37° C. Note small amount of growth on potato medium and its absence on the other two mediums.

purpose. The method is suitable for use in isolating pure cultures of tubercle bacilli from any contaminated sources, such as urine, feces, tissues, and pathologic fluids as well as sputums.

Preparation of Reagents—To prepare 6 per cent sulphuric acid solution, take 3.4 cc of concentrated sulphuric acid (specific gravity 1.84 H_2SO_4 , content 95-96 per cent) and cautiously pour into distilled water to a final volume of 100 cubic centimeters. To prepare 3 per cent hydrochloric acid, 7.1 cc

of concentrated hydrochloric acid (specific gravity 1.18, HCl 35.36 per cent) are diluted to 100 c.c. with distilled water.

Both of these acids have proved suitable.

Preparation of the Medium—Large clean potatoes free from surface defects are cut into cylinders about 3 inches in length and $\frac{5}{8}$ inch in diameter, using for this purpose a cork borer. They are then halved longitudinally. As soon as they are cut, these potato cylinders are soaked in 1 per cent sodium carbonate solution containing crystal violet (the stain added just prior to use since prolonged contact with the sodium carbonate leads to decolorization) in concentration of 1:75,000 or 0.003 per cent. After soaking from one to two hours, they are gently wiped with a clean towel to free them from excess of fluid and are then introduced into a sterile culture tube (6 by $\frac{3}{4}$ inch in size) containing $1\frac{1}{2}$ cubic centimeters of 5 per cent glycerin bouillon, cotton plugged, and sterilized in an autoclave at 15 pounds pressure for at least thirty minutes.

Treatment of Sputum or Tuberculous Material and Seeding—Sputum or tuberculous material is first thoroughly beaten up to a homogeneous pulp and 1 c.c. of the resultant fluid is introduced into a sterile centrifuge tube of 15 c.c. capacity. One cubic centimeter of 6 per cent sulphuric acid or 1 c.c. of 3 per cent hydrochloric acid is added and the contents are thoroughly mixed together. Too much emphasis cannot be placed on the thoroughness of mixing, since the number of contaminations depend greatly upon this. The tube is stoppered with a sterile cork and incubated at 37° C. for thirty minutes with occasional shaking. At the end of thirty minutes the contents are diluted with about 10 c.c. of sterile 0.9 per cent saline solution, well mixed and centrifuged. The supernatant liquid is decanted, and the remaining fluid measuring from about 1 to 2 c.c. is well mixed by means of a sterile pipette and is seeded on the surface of the crystal violet potato cylinder medium. After being cotton plugged and capped with tin foil or with light paraffin impregnation of the cotton, the culture tubes are kept in an incubator at 37° C. Within from two to five weeks a luxuriant elevated growth of tubercle bacilli becomes visible, especially above the surface of the 5 per cent glycerin bouillon.

A NOTE ON KEEPING CULTURE TUBES

Often when spores of molds are abundant in the air these germinate on the surface of the cotton plugs of the culture tubes and finally penetrate down through the stopper into the medium, thus spoiling cultures of tubercle bacilli. In laboratories where large numbers of cultures are being kept over a long time, this loss may become considerable. Frequently during the course of isolating primary cultures or perpetuating cultures of tubercle bacilli, the prevention of mold growth plays an important role in the success of these endeavors. Any method used to prevent the growth of molds must also consider and make provision for retaining the optimum conditions for the growth of the tubercle bacilli. The following several methods were studied because some had been recommended as superior to the usual method of using a plain paraffined cotton plug or a tin foil cap. I. Plain nonabsorbent cotton plug

(a) full-dipped in melted paraffin, (b) half-dipped in paraffin, (c) untreated with paraffin II Nonabsorbent cotton plug previously treated with 1 500 mercuric chloride solution (a) full-dipped in melted paraffin, (b) half-dipped in paraffin, (c) untreated with paraffin III Nonabsorbent cotton plug previously treated with 1 per cent sodium salicylate (a) full-dipped in melted paraffin, (b) half-dipped in melted paraffin, (c) untreated with paraffin IV Effect of naphthalene vapor—untreated with paraffin

In all the cases above except IV, The Study of the Effect of Naphthalene Vapor, eight tubes for each subdivision (namely, four tubes for 5 per cent glycerin agar nutrient medium and four tubes for Petroff's gentian violet medium) were seeded with suspensions of tubercle bacilli One-half of these tubes were kept in a moist can while the other half were kept in a dry can, and the number of contaminations by molds and the rate of growth of tubercle bacilli were noted At the end of the fourteenth week of observation it was concluded that the use of dry cans for storing culture tubes should be recommended as compared to the use of moist cans, since the growth of molds is more abundant and common in the latter at incubator temperature If culture tubes are to be kept for longer than four weeks in the incubator, they should be placed in dry metal cans provided with a tight (not air-tight) cover in order to prevent excessive drying of the medium

In spite of the recommendation by Soparkar¹³ that the cotton be soaked in mercuric chloride or sodium salicylate, it was found that culture tubes plugged with a plain cotton plug gave superior growth to those provided with cotton plug which has been previously treated with mercuric chloride (1 500) or sodium salicylate (1 per cent) The toxic effect of mercuric chloride was very evident, being less so but still perceptible with sodium salicylate The use of naphthalene as an antimold measure also proved unsatisfactory, on account of its deleterious action on the growth of the tubercle bacilli

As a result of a comparison of tests with full-dipped, half-dipped in melted paraffin and untreated cotton plugs, it was found that the cotton plugs half-dipped in paraffin are the most desirable Cotton plugs not treated with melted paraffin also gave good results but tended to result in early drying up of the culture medium as well as in a greater likelihood of contamination with molds Cotton plugs heavily soaked with paraffin cannot be recommended because this not only results in an inferior growth of tubercle bacilli but frequently may result in complete absence of growth, due to the inaccessibility of atmospheric air

SUMMARY

Of the various reagents used for the primary isolation of tubercle bacilli from contaminated tuberculous materials, sulphuric acid and hydrochloric acid were found superior to any other reagents tried, including sodium hydroxide, for destroying the undesirable contaminants present

Standard crystal violet or methyl violet of the American dyes was found serviceable in replacing the prewar Grubler's gentian violet, and in the same concentration, for preparing Petroff's gentian violet egg medium

Crystal violet potato cylinder medium is superior to gentian violet egg medium, Dorsett's egg medium, and other mediums for the cultivation and isolation of tubercle bacilli

A new method is described for the isolation of tubercle bacilli from contaminated tuberculous sources incorporating the use of sulphuric acid (or hydrochloric acid) as a reagent for preliminary treatment of the materials and crystal violet potato medium as a nutrient medium for their growth

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DISCUSSION

Dr Robert A Keilty—One of my first loves was tuberculosis. In 1911, I was able to publish one of the first papers on Petroff's method so I am particularly interested in this subject. The potato medium appears to give a more luxuriant growth than is obtained on Petroff's medium. The cultivation procedure is divided into two parts: the destruction of contaminants by acid or alkali and the continuation of retardation by the introduction of some substance into the culture medium. In spite of all the work that has been done on dyes it is almost impossible to tell what you have as they come to you from the manufacturer. The variation in these dyes is anywhere from 3% to 38 per cent of dye activity. I believe that there has been only one of the aniline dyes actually standardized. That is very important when it comes to knowing the amount of material put into the culture medium. In this method the dyes exert an individual specific chemostatic action.

Dr C E Royce—What is the method of sterilization of the medium?

Dr Corper—The method is as follows: Take an ordinary potato peel, cut to cylinder and divide it in half, wash and dip in 0.003 per cent gentian violet in 1 per cent sodium

carbonate solution for two hours. Then autoclave at ordinary high pressure for from about thirty minutes to one hour, after tubing with 15 cc glycerol broth.

Dr. Frank W. Hartman—Did you try the effect of oxygen and carbon dioxide tension?

Dr. Corper—The effect of carbon dioxide and oxygen upon the growth of tubercle bacilli has been extensively studied in our laboratory, and this was considered in these cultivation studies. Suffice to say briefly that the tubercle bacilli grow best in atmospheric air on most culture mediums, although with lower concentrations of oxygen a small amount of carbon dioxide exerts a beneficial influence, while higher amounts of carbon dioxide may be inhibitory. On the potato medium atmospheric air supplies ample oxygen, and the bacilli grow better than in other gas mixtures.

Dr. George Ives—Will Dr. Corper make a few remarks as to the use of the guinea pig in diagnosis, the time it takes tuberculosis to kill a guinea pig, etc. We often want a quick diagnosis. This subject will be interesting.

Dr. Corper (closing)—It must be admitted that any method requiring a wait of a couple of months to make a diagnosis is fraught with difficulty. This is the objection to the use of the guinea pig. I told you before that the guinea pig is an extremely susceptible animal to inoculation tuberculosis and that it requires only from 10 to 100 bacilli from the ordinary sputum to infect this animal. The question of the importance of the route of infection has led to much discussion. You have heard of the many recommendations and preferences. Some prefer injecting intravenously, others into the mammary gland, some the intraperitoneal route, etc. In our own laboratory we have exhaustively investigated the importance of the routes of infection in the guinea pig as well as the effect of various reagents upon the infection in this animal, bearing in mind its importance for the clinical pathologist. The results have been presented to you at previous meetings of this Society. The guinea pig and monkey are extremely susceptible to inoculation infection with tubercle bacilli as few as 10 to 100 bacilli being sufficient to produce a generalized disease in from two to three months. Therefore, whether you introduce the bacilli intravenously, subcutaneously, or intraperitoneally makes little difference, because of the extreme susceptibility of these animals. Our choice has been subcutaneous inoculation for the animals need not be sacrificed and there is a visible index of infection. The local gland begins to enlarge, and upon incising at the appropriate time, a small amount of pus can be obtained for preliminary diagnostic examination. If acid fast bacilli are found, a tentative diagnosis can be made, and at a late date (after a few weeks more) the guinea pig can be sacrificed and the diagnosis verified by finding a generalized tuberculosis in this animal. The enlargement of the local gland depends largely upon the number of bacilli that were present in the inoculated material, with few bacilli it may require a month or two, while when large numbers are present, the local gland will become visibly enlarged and contain pus in a few weeks.

VASCULAR INJECTION IN PATHOLOGY*

By EARNEST SCOTT, M D, AND ROBERT A MOORE B A, COLUMBUS, OHIO

IN THE past a large number of methods have been developed for the study of the hollow organs of the body, more especially the arteries, capillaries, veins, and ducts of the glands. In connection with studies on the vascular changes in nephritis being carried on in this laboratory, it seemed advisable to correlate these various methods and to deduce from them some general principles which may be followed in any type of injection. These principles and a standardization of technic are of the utmost importance in pathologic studies. In anatomy it is desirable, at least in some cases that the injection be incomplete since any attempt to unravel the total circulation is impossible, while if only a part is shown at one time the relationships can be readily seen and followed. In pathology, if a portion of an organ is not injected, the question immediately arises is this noninjection due to some pathologic change or is it a result of faulty technic?

With this viewpoint in mind a standard technic becomes paramount yet any attempt at standardization is of little value if all the various methods of injection cannot be controlled so that the one standardization applies to all. It is this problem which we have attempted to solve in order that any one pathologic condition may be studied by any or all of the methods available and different views secured of the same process.

TYPES OF INJECTION

The various types of injection and injection masses may be conveniently divided as follows

- 1 Fluid masses to be injected at room temperature
- 2 Fluid masses which are injected in warm, fluid state and are then allowed to set in the vessels
- 3 Masses which are injected as a fluid but which set in the vessel and the tissues later removed by erosion
- 4 Masses which are opaque to the x ray
- 5 Specific stains

The cold masses are the easiest to use but they do not give the beautiful pictures which can be secured with other methods. As such an injection mass Lee Brown¹ recommends 2 per cent soluble Berlin blue in water as giving excellent results. Prussian blue is also good and may be prepared by adding equimolecular proportions of chemicals and diluting so that the result of the mixture (Prussian blue) is in a concentration of 2 per cent. Muller used Berlin blue in 1 per cent glue. Other workers have used injections of two fluids which when they meet in the vessels cause the formation

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of a precipitate Bowman³ thus used a method devised by Kiause consisting of potassium dichromate and lead acetate injected successively and giving a precipitate of lead chromate Bird and Moise⁴ injected a solution containing iron ammonium citrate and potassium ferrocyanide and fixed small pieces in acid formalin The acid in the fixative caused the precipitation of Prussian blue wherever the solution had injected By this method they were able to inject the tubular system of the kidney Kerr and Meffier⁵ have used India ink in concentration of 50 per cent in distilled water for injection of the capillaries of the heart valves We have used 50 per cent India ink in distilled water to inject the bursa about the bundle of His in the beef heart, and secured very excellent specimens The technic of this injection is given by Kaufmann⁶ Milk injections and subsequent staining of the fat globules have been used⁷

Warm injection masses, particularly gelatin carmine, give results which are not excelled by any other one method Gelatin is the usual base of the mass, although starch and other substances have been used This type of injection may be divided into those in which the gelatin is stained before injection and those in which the staining of the injection is accomplished in sections prepared from the organ injected

Gelatin masses are usually colored with Berlin blue or carmine The former is not difficult of preparation⁷ It seems desirable, however, that arterial injections should be in red, the usual method of the preparation of carmine injection masses as given by Mallory and Wright⁷ is rather capricious and of little value when consistent and standard results are desired MacCallum⁸ has recently published a method for the preparation of gelatin carmine in which the excess ammonia was removed from the carmine solution by boiling, before adding to the gelatin Benslev⁹ has prepared carmine in a colloidal state and added this to the gelatin However, in attempting to secure a satisfactory gelatin carmine by a short method it occurred to one of us to use the H electrode to adjust the reaction¹⁰ By this method we have uniformly secured excellent masses and with very little difficulty Robinson¹¹ has secured excellent preparations of the circulation in the spleen by the injection of plain gelatin and later staining with iron hematoxylin

In any of the gelatin masses it is sometimes of advantage to add about 5 per cent of potassium iodide to reduce the gelatin point in order that manipulation need not be carried out at so high a temperature

The celloidin corrosion methods are particularly applicable to the demonstration of gross alterations in the larger vessels, but by careful attention to detail good preparations for microscopic study can also be secured Schnefferdecker¹² first proposed the use of celloidin as the injection mass He dissolved the celloidin in ether and used asphalt as the coloring matter This method has the disadvantage that the specimens shrink considerably Hochstetter¹³ modified the technic by using kaolin to prevent shrinkage The method as used today was developed by Krassuskaya,¹⁴ who dissolved the celloidin in acetone and used camphor to prevent shrinkage Huber¹⁵ has used this method with excellent results to demonstrate the origin of the arteriolar rectae Hinman and Morrison¹ have used the method, substituting

used x ray films for the celloidin in the stronger solutions. Numerous dyes have been used to color the mass, among which should be mentioned cobalt blue, chrome yellow, cinnabar, Victoria blue, Berlin blue, Prussian blue, asphalt, alkanin, and a mixture of crystal violet and brilliant green. The specimens in which Victoria blue is used have the advantage that they may be preserved in a dry condition. Our experience with the celloidin method has been very satisfactory and the formulas which have been used are as follows:

For the pelvis of the kidney

Acetone	100 cc
Celloidin	20 gm
Camphor	16 gm

For the larger blood vessels

Acetone	100 cc
Celloidin	10 gm
Camphor	8 gm

For the glomeruli

Acetone	100 cc
Celloidin	2.75 gm
Camphor	2.0 gm

The exact strength of the latter solution is adjusted to a known viscosity as given later in this paper. All solutions are prepared from pyroxylin, Du Pont. We have found scarlet R satisfactory as a red stain and methyl green as a bluish green. In place of these dyes one may use ordinary oil pigments with equally satisfying results. The concentration of scarlet R should be greater in the thin solution than in the thick, a concentration of 0.3 per cent has been found satisfactory for the thin solution.

After the injection the tissues may be removed by either pepsin 1:3000 in 0.4 per cent HCl or 75 per cent HCl. After from twenty four to forty eight hours the softened parenchyma may be removed with a gentle stream of water. For preservation of the specimen the solution recommended by Hinman et al¹ consists of distilled water 100 cc, formalin, 2 cc and glycerin 20 cc. Specimens may also be shown to advantage by mounting in gelatin, according to the technique of Delephne¹⁷. Such specimens mounted so that they may be viewed by transmitted light give striking pictures of the richness of capillary circulation.

Ghorey¹⁸ has used the corrosion method to study the circulation of chronic nephritis and the coronary arteries. Instead of celloidin he injected Babbitt metal at 85° C after irrigation with Adler's salt solution at 85° C for 90 seconds. His preparations as illustrated give in graphic form the relation of circulation to the severity of chronic nephritis in a most excellent manner.

The first attempts with x ray opaque materials was with metallic mercury. More recently two types of injection have been developed, one using a cold fluid mass and another using a warm gelatin or lipoidal base. Beck¹⁹ used a suspension of bismuth subnitrate in petrolatum. Katzenstein²⁰ suspended the bismuth subnitrate in gelatin. Lee Brown¹ recommends a suspension of finely divided barium sulphate in 50 per cent potassium iodide. We

have used the latter method with good results. In an attempt to secure a finer injection we have prepared a solution of potassium iodide in water and glycerin, but the richness of circulation prevented the securing of any roentgenograms of value. Our impression of this method is that it is very satisfactory for the larger vessels, especially if stereoscopic roentgenograms are made, but that for a detail study, it is of no value.

Specific stains that have been used are silver nitrate and Janus green. The silver nitrate methods are so capricious that they need not be discussed here. Janus green as a specific stain for endothelium has been utilized by Bensley with brilliant results.²¹ Recently MacCallum⁸ from Bensley's laboratory has published results secured by using a combination of Janus green and gelatin carmine. This method gives the clearest picture of the finer circulation of any that we have used. The Janus green B in aqueous (1:10,000) solution injected into the arterial tree continues on into the veins. After injection the organ is allowed to stand until the dye has been largely changed to a red color, then the Janus green is washed from the vessels by warm saline solution, and the vessels are slowly irrigated with 2.5 per cent ammonium molybdate to fix the blue dye remaining, then gelatin carmine is injected in the usual manner. In this way the endothelium of the arteries and glomeruli are stained a greenish blue while all the vessels, arteries and veins, are filled with the red gelatin. Thus, the unraveling of the circulation into arteries and veins is materially facilitated. This method is only applicable to living tissue.

THE TECHNIC OF INJECTION

The steps in the injection of an organ may be divided conveniently into first, preparation of the tissue for injection, second, the actual injection, and third, the after treatment and preparation for demonstration.

The preparation of the tissue involves at least two types of manipulation: first, the removal of the blood from the vessels, and second, the securing of a closed vascular system.

For the irrigation of the vessels to remove the blood, Ghorey¹⁸ used diophsical fluid. Hinman et al.¹ recommended saline. We have used both normal saline and tap water and have not been able to detect any material difference. When one is working with a supravital stain, as Janus green, it is advisable to use warm normal saline, but for all ordinary injections one may simply connect a cannula in the artery or vein to a city water hydrant and allow the water to flow through the vessels until the return flow is clear and the organ a pale gray color. The pressure used is approximately 300 mm Hg. In the preparation of kidneys for celloidin injections, it has been our policy to use the above technic, with the water flowing at a rate which allows about 150 gallons to flow through the capillary bed in twelve hours.

Clot dissolvers, such as 10 per cent sodium sulphate, have been recommended, but in our experience they are of little value. If within an hour the vascular tree is not free of blood, it probably never will be, regardless of the pressure or solution that is used.

Other workers recommend the use of vasodilators. In the case of animals, the nitrates have been administered before death to secure vasodilatation. We have used this procedure on several rabbits and failed to secure better results. With organs removed after death, Gross recommended the injection of 10 per cent potassium sulphocyanide to release the postmortem rigor of the vessels. Again, this procedure has not yielded better results in our hands.

When the vascular tree has been cleared of all blood, the next concern is to make it a closed system, open only at the arterial and venous ends. Hinman et al¹ make particular mention that no air should be allowed to enter the vessels, and in accordance with this idea in our early work water was injected and the cut vessels were found by the jet of water springing from them and were ligated. This is a laborious process and it is difficult to locate all the vessels. To circumvent this, we decided to use air. In this technique the organ is immersed in water and air under a head of 200 mm Hg is forced into the vessels. In this manner the presence of severed vessels is shown by a stream of air bubbles and in a few moments the ligations can be completed. As yet we have not seen any untoward results from the injection of the air. In other words, we secure just as good results after filling the vessels with air as when precautions were taken to prevent the entrance of air.

With the organ ready for the actual injection one is next concerned with the injection mass, the types and preparation of which have been discussed in the first part of this paper. It only remains to attempt a standardization which will include all the different types.

It is a well known fact that the rate of flow of a fluid in a closed tube depends chiefly on two factors—the diameter of the tube and the viscosity of the fluid. Theoretically then one should be able either to reduce the diameter of the tube or to increase the viscosity of the fluid to a point where the fluid will not flow. In dealing with the organs of the body the size of the tube (capillaries) is fixed for that particular organ, and if one desires to cause a cessation of flow of the fluid through this vessel, the viscosity must be increased. Following this principle, we have carried out experiments to determine the concentration of celloidin in acetone which when injected into the kidney will have a viscosity such that it will fill the glomerular capillaries but its viscosity is just above the point at which it will flow through the efferent vessels of the glomerulus. The results of these experiments were that a solution with a relative viscosity of 16.5 (compared to water) possessed this property. To confirm the applicability of this principle, we have prepared gelatin and adjusted its viscosity to this point and injected other human kidneys. The results were that the gelatin carmine passed to the efferent vessels but not beyond. (NOTE: Gelatin possesses the property of hysteresis hence the time elapsing from the melting of the mixture to the measurement of its viscosity must be considered.) The approximate strength of the celloidin mass of such viscosity is 2.75 per cent celloidin and 2 per cent camphor in acetone. Of course this principle does not apply to any mass in which solid particles are suspended, nor does it of

necessity apply to other organs of the same or other species of animals, but it does make possible uniform injections where numerous specimens of the same organ are to be compared and an attempt made to show pathologic change. In using cold fluid masses, glycerin may be added to the mass to increase the viscosity.

The next problem is the pressure to be used and how this pressure is to be applied. This may conveniently be divided into cases in which only a part of the entire length of the vascular tree is to be injected and those cases in which the entire circulation, arteries, capillaries and veins, is to be injected. In either case it will be found convenient to use a tank of compressed gas with an adjustable reducing valve attached, so that any pressure from 50 to 600 mm of Hg may be secured and this pressure kept constant for hours. In general the following pressures will be found satisfactory:

Arterial system	600 mm Hg
Venous system	250 mm Hg
Ductal system	20 to 80 mm Hg

The maintaining of these pressures over long periods of time is important. In the case of partial injection, say a celloidin injection up to and including the glomeruli, we have found that for consistent and satisfactory results the pressure should be maintained for twenty-four hours. The organ is immersed in water, and the thin celloidin injected under a constant pressure of 600 mm of Hg for six hours. At the end of this time the thin solution is replaced by the 10 per cent solution and the injection continued for eighteen hours longer. We believe that by this technique, with a definite obstruction at the efferent vessel, the celloidin is pushed in all the finer vessels, which it would not enter if the pressure were only maintained for a short time. Further, by immersing in water, the acetone is attracted to the water from the celloidin, thereby precipitating the celloidin. Thus, a little space is left, and as the pressure is still present, more celloidin enters. By this process one secures an accurate cast of the lumen of the blood vessels.

When the injection mass passes through the entire system, a different problem is presented. In this case there is no obstruction to the free flow and if, as usually occurs, a certain pathway through the organ offers less resistance, this part will inject perfectly, and the remainder will inject only in parts. An obstruction could easily be offered by clamping the vein, but the high pressure used in arterial injection would soon rupture the veins and spoil the specimen. In our work we have used intermittent clamping of the vein, thus for a few moments causing an increase of pressure in the vascular tree and forcing the injection mass into the smaller vessels, then releasing and repeating the process several times at short intervals. We believe that this one point will determine, at least in the majority of cases, whether one secures a complete capillary injection or only a useless partial injection.

With the actual injection completed, the question of proper demonstration of the results must be considered. With cold and warm injections the organ is fixed in alcohol or formaldehyde, and sections are prepared in the

usual manner. The warm injections are usually first placed in ice water to set the gelatin, and it has been our policy to place the entire organ in ice cold 10 per cent formaldehyde after injection and leave in the ice box twenty four hours. With this technique the organ may be cut after twenty four hours without danger of loss of the injection mass from the smaller vessels. Sections from 10 to 50 microns in thickness may be used or slabs from 1 to 3 mm may be cut and cleared in aniline oil.

With the corrosion specimens the technique of corrosion and mounting has already been considered. In case one desires to preserve the soft parts and only the larger vessels have been injected the organs may be cleared by any of the usual methods and the vessels seen in their relationship to the soft parts.

DISCUSSION

The use of injection methods in pathology is twofold: first for purposes of research, and second, for the clear and concise demonstration to students of vascular changes in an entire organ which cannot be demonstrated by any other method.

Its use in research is already assuming such proportions that we cannot review all the work at this time but only point out for consideration the organs of the body in which it has been used.

McIndoe and Counsellor⁸ have studied the changes in the portal system in atrophic cirrhosis and in the ductal system in cases of long standing obstructive lesions. They have also investigated the supposed dilatation of the biliary passages in cases of cholecystectomy. In portal cirrhosis they have demonstrated that very little of the portal blood passes through the liver. The question of liver function in these cases must be of importance.

Hinman⁷ has investigated extensively the question of hydronephrosis and the related problem of pelvic venous backflow.

Work on nephritis by Gross^{16, 20} has already been mentioned. Recently he has devoted an entire book²⁴ to the question of encrustation in the heart valves and apparently has placed the theory of embolic origin of endocarditis on a much firmer foundation.

Ghorey¹⁸ has determined the capacity of the coronary circulation in relation to age, and concludes that although the heart increases in size with age, there is not a proportionate increase in the capacity of the coronary arteries.

Terry²⁵ has injected the circulation in adenomas of the thyroid. He has demonstrated that the veins in these adenomas are very thin walled and will not withstand even 100 mm Hg pressure without rupturing. This fact is offered as a possible explanation of the frequency of hemorrhage in this type of thyroid disease.

Sampson⁶ has prepared many injections of fibroid uteri and demonstrated the relationship of vascular supply to these tumors.

SUMMARY

- 1 The methods available for the study of organs by injection of the vessels or ducts are reviewed
- 2 The general principles involved in the technic of securing a complete and satisfactory injection are reviewed
- 3 A method of standardization of injection masses by measurement of viscosity is given
- 4 A short review of the possibilities of the use of these methods in pathology is given

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THE RELATIVE DIAGNOSTIC VALUE OF THE LEVINSON TEST AND THE GLUCOSE CONTENT IN CEREBROSPINAL FLUID*

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IN A PREVIOUS communication¹ it was pointed out that the normal range of the glucose content of the cerebrospinal fluid lies between 60 and 90 mg. Values less than 60 mg. are, in the majority of instances, associated with inflammatory processes in the central nervous system, and, in the absence of pyogenic organisms in such fluids, a tentative diagnosis of tuberculous meningitis can be made.

The validity of this provisional diagnosis based on a consideration of the glucose content alone, must be established by correlation with other tests and clinical findings. It is generally recognized that the present means of making a definite diagnosis of tuberculous meningitis are limited. The characteristic clinical picture is seldom present in the early stages of the disease; the finding of the organisms in the smear is attended with uncertainty, and cavy inoculation entails too much delay. Hence, it becomes of interest to ascertain the value of any new test which may have diagnostic significance in conditions simulating tuberculous meningitis.

The Levinson test² commends itself for investigation because of its simplicity and the rapidity with which results may be read. In this test there is precipitation of proteins as albuminates when the spinal fluid is treated with certain metallic salts and as insoluble salts when it is treated with weak organic acids. The procedure as outlined by Levinson is as follows: One cubic centimeter of spinal fluid is placed in each of two tubes. To Tube 1 is added 1 cc. of 1 per cent mercuric chloride. To Tube 2 is added 1 cc. of 3 per cent sulphosalicylic acid. The contents of the tubes are mixed, and the tubes stoppered and allowed to stand for twenty-four hours. The column of precipitate is then measured in millimeters.

Levinson maintains that in cases of purulent meningitis the height of the column of precipitate after the addition of sulphosalicylic acid is three times that following the addition of mercuric chloride, while in cases of tuberculous meningitis the bichloride precipitate is twice that of the acid.

Pons and Fletcher³ recently reported on a relatively small series of spinal fluids; they were favorably impressed with the value of the Levinson test. They pointed out, however, that the test is not pathognomonic of tuberculous meningitis, since in their experience in a series of twenty-four spinal fluids from cases of syphilis of the central nervous system, 25 per cent of the positive reactions were false.

Our report is based on the study of ninety-eight spinal fluids from proved

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cases of tuberculous meningitis, syphilis of the central nervous system, purulent meningitis, aseptic meningitis, and varied conditions involving the central nervous system

METHOD

The method of Folin and Wu, with precipitation of the proteins by the tungstic acid reagents, was used for the quantitative sugar determinations. When the sugar content was less than 25 mg, twice the amounts of filtrate were used, or the dilution of the unknown was made up to 125 c c instead of 25 c c, to facilitate the reading. All aseptic spinal fluids with a sugar content of less than 60 mg were inoculated into guinea pigs to determine the presence or absence of bacilli of tuberculosis. All globulin estimations were made by the Noguchi method using 0.2, 0.5, and 0.1 c c. The Levinson test was carried out as described and in many instances a duplicate test was made using double concentration of the reagents as suggested by Pons.

RESULTS

Table I shows the results obtained with twenty-three spinal fluids from seventeen proved cases of tuberculous meningitis. The Levinson test was positive in twenty cases (86 per cent), that is, the precipitate with bichloride was twice as high as that with acid. All fluids showed a higher precipitate with mercury, the range being from 1.71 to 4.51 with an average ratio of 2.71. In two of the fluids a precipitate was obtained by using the test solutions in double strength.

The test proved an aid in diagnosis in Case 3 in which the glucose estimation (80 mg) of the first specimen was misleading. Later specimens

TABLE I
SPINAL FLUID OBSERVATIONS IN TUBERCULOUS MENINGITIS

CASE	HEIGHT IN MM				RATIO	GLUCOSE, MG	CHLO RIDES, MG	CELL COUNT	GLOBULIN, GRADE	EXAMINA TION OF SMEAR	GUINEA PIG INOC ULATION
	SUL 3%	HgCl ₂ , 1%	SUL 6%	HgCl ₂ , 2%							
1	7	15	7	20	1.21	37	620	120	4+	Negative	Positive
2	4	11	4	13	1.27	37	645	40	2+	Negative	
2						26	655	280	3+	Negative	
2	3	10	4	12	1.33	29	665	189	4+	Positive	Positive
3	3	7	4	8	1.23	80	580	20	1+	Negative	
3	5	18	5	17	1.36	30	625	30	4+	Negative	Positive
4	3	0	2	8	3.0	40	665	50	4+	Negative	
4	3	1	4	7	3.1	51	710	160	2+	Negative	Positive
5	6	7	8	9	1.11	54	630	274	2+	Negative	
5	5	15	8	22	1.3	74		240	3+	Negative	
5	7	13	7	15	1.18	30	610	260	3+	Positive	Positive
6	1	15			1.15	28		165	1+	Negative	Positive
7	1	2			1.2	22		98	1+	?	Positive
8	2	9			1.45	28	640	430	2+		Positive
9	4	10			1.25	5	570	500	4+	Positive	Positive
10	2	7			1.35	37	650	278	2+	Negative	Positive
11	3	10			1.33	18		192	1+	Negative	Positive
12	5	15			1.3	5		20	1+	Negative	Positive
13	6	14			1.23	11		400	2+	Negative	Positive
14	4	9			1.22	25		42	2+	Negative	Positive
15	5	10			1.2	0		45	2+	Negative	Positive
16	5	14			1.28	26		65	2+	Positive	Positive
17	5	13	7	21	1.26	26	585	190	2+	Positive	Positive

TABLE II
SPINAL FLUID OBSERVATIONS IN TURBULENT MENINGITIS

CASE	HEIGHT IN MM				RATIO	GLUCOSE MG	CHLORIDES MG	CYTA COUNT	GLOBULIN GRADE	ORGANISM FOUND	
	SUL 3%	HQCL 1%	SUL 6%	HQCL 2%						IN SHEAR	ON CLTURE
1	17	10	22	22	1 1	0	700	1000	4+	Pneumococcus	Pneumococcus
2	4	8	4	12	1 2	61	700	1200	4+	Pneumococcus	Pneumococcus
3	4	9	4	14	1 2 2	20	700	1400	4+	Pneumococcus	Pneumococcus
4	12	2	13	3	4 3 1	0	600	2000	4+	Pneumococcus	Pneumococcus
5	10	0	0	0	3 1 0	0	600	1400	4+	Pneumococcus	Pneumococcus
6	0	4	2	10	0 0 0	68	680	2400	3+	Gram negative bacillus	Bacillus influenzae
7	8	0	5	3	1 2	5	640	1600	2+	Streptococcus	Hemolytic streptococcus
8	5	0	9	0	1 1 1	0	640	400	4+	Streptococcus	Hemolytic streptococcus
9	5	0	4	7	1 1 1	6	640	700	4+	Streptococcus	Hemolytic streptococcus
Serum	19	3	4	7	1 1 6	0	640	26000	3+	Diplococcus	Meningococcus
Serum	17	10	10	23	2 3 1	42	675	3000	3+	Diplococcus	Meningococcus
Serum	14	10	10	17	1 1 1	66	680	1000	2+	Diplococcus	Meningococcus
10	8	18	10	28	1 1 1	74	710	500	2+	Negative	Sterile
				20	1 2 2	0	621	11000	4+	Diplococcus	Meningococcus

showed more characteristic glucose values. In Case 5 subsequent tests showed little difference in the height of the precipitates from the first specimen. Later specimens yielded ratios typical of tuberculous meningitis. Obviously a diagnosis based on the findings in the first fluid obtained in the early course of the disease may be erroneous. This observation was recently published by Fremont-Smith and Ayei.⁴ The glucose content in twenty-two fluids (95 per cent) ranged from 0 to 54 mg, the average being 29 mg. It is interesting to note that the range for chlorides is below 700 mg.

Table II shows the results obtained in fifteen spinal fluids from ten cases of purulent meningitis. Four of these fluids (26 per cent) yielded false reactions in the Levinson test. Seven fluids (46 per cent) gave a heavier precipitate with acid, the average ratio being 1.26. The glucose content in Cases 2 and 6 in the first fluid was misleading, but in later specimens it was typical of purulent meningitis. In Case 8 the glucose value of 65 mg was not typical. The chlorides were variable.

TABLE III
SPINAL FLUID OBSERVATIONS IN ASEPTIC MENINGITIS*

CASE	HEIGHT IN MM				RATIO	GLUCOSE, MG	CHLORIDES, MG	CELL COUNT	GLOBULIN, GRADE
	SUL 3%	HGCL, 1%	SUL 6%	HGCL 2%					
1	4	7	0.4	1.5	1.17	60	725	2200	2+
2	9	3			3.1	25	660	2800	4+
3	4	8			1.2	52		3170	4+
4	3	0	2	8	3.0	41	665	50	4+
5						57	690	7300	4+
5			5	10	1.2	58	745	4980	4+
6	8	15	11	26	1.18	13	675	7200	4+
6	10	20	11	31	1.2	45	645	6800	4+
6	15	4	25	11	3.71	48	665		
6	15	3	13	22	5.1	83	675	14000	

*Smears, cultures, and guinea pig inoculations were negative in each case.

Table III shows the observations in ten spinal fluids from six cases of aseptic meningitis. The Levinson test was done in the case of nine of these fluids, three of which showed false reactions. In only three was the 1.3 ratio evident which Levinson reports to have obtained in nontuberculous cases. The cell counts ranged from 50 to 14,000 for each cubic millimeter, and the glucose from 13 to 83 mg, the average was 48 mg. The final diagnosis in these cases was dependent on the subsequent clinical course of the patients, the findings at necropsy, and the cavy inoculations. This is the group of cases most difficult to differentiate from tuberculous meningitis. The clinical symptoms, such as headache, vomiting, and even rigidity of the neck are common in both conditions, and the spinal fluid findings in this group are identical with those found in cases of tuberculous meningitis. It is only when all possible clinical data are correlated with the spinal fluid findings that a correct diagnosis can be established. The following report of a case will serve to illustrate the point.

A married woman, aged nineteen, came to hospital complaining of marked frontal headache, nausea, vomiting, and dizziness, of one week's duration. Since childhood there had been a discharge from the ear. Physical examina-

TABLE IV
SPINAL FLUID OBSERVATIONS IN SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

CASE	HEIGHT IN MM				RATIO	GLUCOSE MG	CHLORIDES MG	CELL COUNT	GLOBULIN GRADE	WASSER MANN REACTION
	SUL 3%	HGCL 1%	SUL 0%	HGCL 2%						
1	3	6			1 2	63	715	30	3+	4
2	7	6	5	4	1 1 1	83	72	0	0	4
3	2	4	2	4	1 2	6	701	38	2+	4
4	4	6	3	5	1 1 1	62	730	7	2+	4
4	4	10			1 2 5	58	755	60	3+	
5	4	8			1 2	86	700	40	2+	4
6	1	1			1 1	64	760	0	0	3
7	3	7			1 2 3	78	780	60	4+	4
8			2	4	1 2	81	745	8	0	1
9	3	4			1 1 3	73	785	6	1+	4
10			3	8	1 2 6	61	790	190	3+	4
11			4	8	1 2	60	753	85	2+	4
12	2	3			1 1 5	77	740	0	1+	4
13	1	3	1	2	1 3	67 5		60	3+	4
14	1	4			1 4	60		0	1+	-
15	1	4			1 4	70		10	0	4

TABLE V
SPINAL FLUID OBSERVATIONS IN VARIOUS CONDITIONS

CLINICAL DIAGNOSIS	HEIGHT IN MM				RATIO	GLUCOSE MG	CHLORIDES MG	CELL COUNT	GLOBULIN GRADE
	SUL 3%	HGCL 1%	SUL 6%	HGCL 2%					
Meningismus	2	2			1 1	83	680	10	0
Meningismus	0	0			0 0	71	705	4	0
Meningismus	0	0			0 0	77	650	4	0
Meningismus	0	0	0	2	0 0	72		40	0
Meningismus	3	1	3	2	3 1	84	760	0	0
Meningismus			2	2	1 1	90		0	0
Psychoneurosis			1	3	1 3	72	800	4	1+
Hysteria			3	5	1 1 6	90		0	0
Fracture of skull	3	3	3	9	1 1	133	615	6	0
Fracture of skull	1	3			1 3	91	740	0	0
Fracture of skull	0	1	0	4	0 1	70	735	0	0
Neuritis	2	4			1 2	106		0	0
Neuritis	1	1	1	1	1 1	69	770	8	0
Apoplexy	2	2	2	2	1 1	68	720	4	1+
Ghoma of brain	4	3			1 3 1	61	530	34	2+
Ghoma of brain	3	10	2	9	1 3 3	76		0	0
Uremia	2	2			1 1	126		0	0
Encephalitis	1	1	5	4	1 1	75	760	10	0
Tetanus	1	1			1 1	77		3	0
Tetanus	3	4	2	6	1 1 3	83		0	0
Renal abscess	0	0	0	0	0 0	66	745	3	0
Headache	3	6	2	7	1 2	63	740	0	0
Perniciosa anemia	3	7			1 2 3	56	700	0	0
Cholecystitis			3	5	1 1 6	71		0	0
Suspected lues	3	4			1 1 3	70		0	0
Suspected lues	2	0	2	0	2 0	79	740	0	2+
Suspected lues	1	3			1 2	70	710	0	0
Suspected lues	0	0	2	0	0 0	73		3	0
Suspected lues	2	5			1 2 5	62	770	4	0
Suspected lues	1	3	2	4	1 3	78		0	0
Suspected lues	1	1			1 1	84	750	0	0
Suspected lues	1	1			1 1	75		0	0
Suspected lues	1	2	1	3	1 2	81	700	8	0
Suspected lues	2	5	2	7	1 2 5	80		0	0

Cultures and Wassermann reaction were negative in each case

tion revealed a divergent squint and choked discs, more marked on the right, there was also a slight purulent discharge from the left ear but no evidence of mastoid blocking or edema. The leucocytes numbered 7,600, and the hemoglobin was 75 per cent, urinalysis was negative and her blood was sterile on culture. Lumbar puncture revealed a cloudy fluid, the cell count was 2,800, chiefly polymorphonuclears, globulin 4+, and glucose 25 mg for each 100 c c. The Levinson test showed a precipitate of 9 mm with mercuric chloride and 3 mm with sulphosalicylic acid, a ratio of 3:1. No bacteria were found in stained smears of the sediment, and cultures in various media were sterile. A guinea pig was inoculated.

A roentgenogram of the head revealed digitation throughout the cranium. The pneumatic cells were obliterated on the left side.

In view of these observations the changes in the spinal fluid were considered secondary to brain abscess. Exploratory craniotomy revealed a cerebellar abscess, this was verified at necropsy, and later the cavity inoculation proved negative for bacilli of tuberculosis.

In Table IV are shown observations on sixteen spinal fluids from fifteen cases of syphilis of the central nervous system, fourteen (87 per cent) showed a higher precipitate with mercuric chloride. Of these, eleven (68 per cent) showed the 2:1 ratio considered pathognomonic of tuberculosis. The range of glucose content was within normal limits, from 53 to 86 mg, with an average of 68 mg. The chloride content ranged from 700 to 780 mg for each 100 c c.

Table V represents a group of thirty-four spinal fluids obtained in cases of various conditions in many of which the central nervous system may be indirectly involved. Eleven (32 per cent) yielded false reactions in the Levinson test, sixteen (47 per cent) showed higher precipitate with mercuric chloride, three (8 per cent) higher precipitate with the acid, five (14 per cent) no precipitate, and ten (29 per cent) the same depth of precipitate with both mercuric chloride and sulphosalicylic acid. The glucose content ranged from 56 to 133 mg, an average of 74 mg. The chloride content ranged from 530 to 800 mg for each 100 c c.

SUMMARY

In the ninety-eight spinal fluids examined, 32 per cent yielded false reactions. In 100 per cent of the proved cases of tuberculous meningitis, there was a heavier precipitate with mercuric chloride, and in 90 per cent of these the ratio was 2:1 or higher. In the first specimens of spinal fluid, results in the glucose content as well as by the Levinson test were sometimes at variance with those obtained from later specimens. In conditions other than tuberculous meningitis the Levinson test is variable, this is particularly true in cases of syphilis of the central nervous system. Fluid showing a heavier precipitate with mercuric chloride, in the ratio of 2:1 or higher, may or may not be from a case of tuberculous meningitis. Fluid showing a heavier acid precipitate in our experience does exclude tuberculous meningitis.

CONCLUSIONS

1 The percentage of false reactions in the Levinson test is too high to be of positive diagnostic aid in tuberculous meningitis

2 Low glucose values are not diagnostic of tuberculous meningitis, even in the absence of pyogenic organisms

3 The glucose content of the cerebrospinal fluid yields valuable diagnostic information when correlated with all possible available clinical data

The chloride content in tuberculous meningitis is less than 700 mg and not constant in other conditions

REFERENCES

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²Pons C A and Fletcher T A The Levinson Test and Other Laboratory Studies in Tuberculous Meningitis JOUR. LAB AND CLIN MED 1927 xi 514
³Fremont Smith, F and Ayer J B Cerebrospinal Fluid in Differential Diagnosis Jour Am Med Assn 1927, lxxviii, 1078 1079

LAMOTTE WUTH BROMIDE COMPARATOR

By W A TAILOR,* PH D BALTIMORE, MD

IN THE treatment of patients who are given bromides either as a sedative or for epilepsy, it is of extreme importance that the dosage be so regulated that bromide intoxication be avoided. Such intoxication manifests itself either as deliria or as other mental or neurologic symptoms.

Careful work has shown that blood bromides are regulated in large part by salt (sodium chloride) intake, therefore, the dosage of bromide cannot be relied upon, unless it is studied in relation to the actual amount of bromides which reach the blood stream.

The LaMotte Wuth Bromide Comparator was developed in cooperation with Dr Otto Wuth, of the Henry Phipps Psychiatric Clinic Johns Hopkins Hospital, using the method worked out by Wuth¹ for determining the actual bromide content of the blood serum. The test is so simply arranged that it requires very little technical skill and can be used as a routine laboratory test. At the same time it gives results that are sufficiently accurate for all clinical purposes.

The test is based on the fact that solutions of bromides give variable colors with gold chloride, the color depending on the bromide content. Before this test can be applied to blood serum, however, the serum must of course be coagulated and filtered. The test is then made on the clear filtrate, and the color secured is compared with standards representing definite amounts of sodium bromide.

Chemical Director LaMotte Chemical Products Company
 Jour Am Med Assn 1927 lxxviii 9013

The comparator, as shown in Fig 1, contains 50 c c of trichloroacetic acid reagent, 50 c c of gold chloride reagent, seven bromide color standards, representing 75, 100, 125, 150, 175, 200, and 300 milligrams of sodium bromide per 100 c c of serum, three test tubes, and two 0.4 c c pipettes fitted with nipples. These pipettes should be kept in the reagent bottles, since the nipples act as seals. Care should be taken that the trichloroacetic acid reagent does not come in contact with the rubber nipple, as it will disintegrate it.

The top of the box contains three slots and is used as a comparator block in making measurements. A piece of etched glass is fastened to one side of the block, directly over the three slots, by means of a brass holder. This is a great aid in making accurate measurements, as it eliminates the reflection of outside objects in the tubes. The block should be held so that the etched glass is on the side facing the source of light.

A determination of the bromide content of the blood serum is carried out as follows. Draw 10 c c of blood from a vein, and allow it to clot. To 2 c c of the thus gained serum, add 4 c c of distilled water, then 12 c c

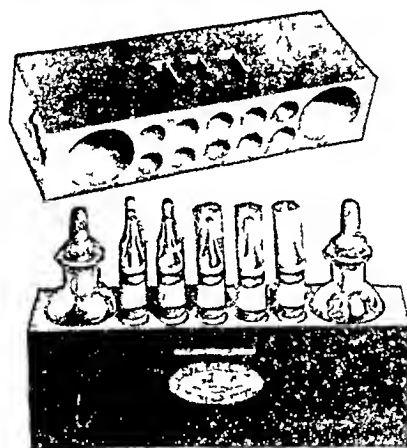


Fig 1

(3 x 0.4 c c) of Solution 1 (Trichloroacetic acid). Shake, allow to stand thirty minutes, and filter. The filtrate must be clear, if it is not, filter again. To 2 c c of the filtrate, add 0.4 c c of Solution 2 (gold chloride), shake thoroughly, place in the comparator, and compare with the color standards. Comparison is best made by placing the test sample in the middle hole opposite the slots next to the ground glass plate, and then inserting color standards in the holes on either side, always making sure that the standards so placed are consecutive, that is 75 and 100, 100 and 125, 125 and 150, etc. If an exact match is obtained, the sodium bromide content is read off directly from the standard. If, however, the color of the sample lies between those of two consecutive standards, the intermediate value must be estimated. The numbers on the color standards give the sodium bromide content of the blood serum expressed in mg per 100 c c. A content of less than 100 mg per 100 c c probably will be insufficient for epileptics. A content of over 150 mg per 100 c c will denote an approach to bromide intoxication.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

VARICOSE ULCER Mechanism of Its Causation and Cure with Notes on the Associated Pain and Papillary Inequality Byrne J. *Am Jour Med Sc* October 1926 *clxxii* No 4, p 553

The immediate primary mechanistic factor in varicose ulcer is incompetency of the superficial vein valves which, in the erect or sitting posture permits the unsupported columns of blood to add their pressure to the normal intravenous pressure thereby counterbalancing or even overbalancing the intracapillary pressure the net result being impairment of blood and lymph flow and increased tissue vulnerability.

Anything that impedes the return flow of blood to the heart from the lower extremities may act as a remote primary mechanistic factor by inducing blood and lymph stasis and dilatation of the veins.

Secondary mechanistic factors are infection, cicatrized ulcer margin enforced inactivity, endocrine dysfunction and impaired metabolism—general and local.

The fundamental indication in surgical or nonsurgical treatment is restoration of the local circulation by removal or support of the long column of venous blood which impedes or halts the capillary flow.

Many surgical and semisurgical methods of treatment of varicose ulcer are unsound in principle; excision and multiple ligation are sound in principle and applicable in certain cases but they should always be supplemented by general and local prophylactic measures.

In severe, neglected cases treatment is best begun with recumbency and elevation which offset the immediate primary mechanistic factor.

In the majority of cases ambulatory treatment with compression bandages effects a rapid cure which may be kept permanent by prophylactic measures.

Study of the pain and pupil changes occurring in leg ulcer helps to a better appreciation of the sensory and pupil phenomena found in cardiac and other visceral disorders.

Endocrine treatment is indicated at one stage or another in almost every case of chronic ulcer, somatic or visceral.

Physical exercise is indicated as a cardiovascular tonic and as the best mobilizer and dispenser of the internal secretions.

ECZEMA AND PROTEIN METABOLISM Critical Investigation of the Relation of the End Products of Protein Metabolism to Eczema and Kindred Disorders Michael J. C. *Arch Dermat and Syph* September 1926 *xiv* No 3 p 294

Blood estimations of uric acid in eczema and kindred disorders are of doubtful value as evidence of a uratic pathogenesis of some cases of these diseases.

The improvement in eczema following institution of a purin-free diet can be construed in other ways than by its effect on the blood uric acid content.

Uric acid, urea and creatinine are not dermal irritants. In two persons a moderate erythematous reaction was evoked but only to concentrated solutions of uric acid and urea.

These metabolites have no demonstrable photosensitizing effects or at least, render the human skin mildly photosensitive only in a very small number of persons, and then only when in strong concentration.

Increasing the uric acid content of the blood by injections of lithium urate intravenously in two cases of severe acute eczema produced no change in the subjective or objective symptoms of the disease.

MERCUROCHROME Mercurochrome 220 Soluble in Malaria, Enbanos, F Jour Philip pine Med Assn, July, 1926, vi, No 7, p 215

Report of the use of merurochrome intravenously in the treatment of malaria in seven lepers The dose of mercurochrome was 0.003 gm per kilogram continued in an average of 17 cc of 1 per cent solution, as a rule, only one injection being given

In none of the cases were smears negative for parasites after the injection, though a marked symptomatic improvement (subsidence of temperature and chills) was noted in five cases

ECZEMA AND URIC ACID The Behavior of Injected Uric Acid in Patients with Eczema, Michael, J C, and Nichols, H O Arch Dermat and Syph, September, 1926, xiv, No 3, p 308

In a previous article, Michael reported a study of the relation of uric acid to eczema In that article, two cases of eczema were reported in which intravenous injections of lithium urate were given The purpose of the intravenous injection procedure was twofold first, to determine the effect on the symptoms of the disease of artificially raising the blood uric acid content, and, second, to study the fate of injected uric acid in this disease As disturbance of uric acid metabolism has been considered an important cause of eczema, it seemed worth while to determine whether patients with eczema actually showed any significant variation from the normal in their behavior to injected uric acid The present report, therefore, is chiefly concerned with this biochemical phase of the question. In that respect it amplifies and supplements the previous report

In the five cases here recorded, it has been shown definitely that artificially raising the blood uric acid level by intravenous injection of this metabolite has produced no enhancement of subjective or objective symptoms of eczema. As a matter of fact, in Case 5 this procedure was succeeded by definite improvement In this instance the injection was followed by a chill and fever, and this may have been the important factor in the clinical improvement Of course, other conditions such as rest and diet, may have entered the picture

From the biochemical aspect, the blood uric acid data were in accord with the results obtained in normal persons by previous investigators In every instance the injection was followed by an immediate rise in the blood uric acid level with a subsequent slow but steady fall, except in Case 5, in which this uniform decline did not occur In this case the blood uric acid level may have been influenced by a febrile reaction which developed soon after the reaction

The total recoveries of uric acid varied within moderate limits, as was to be expected The recoveries are comparable to those of Fohn and his collaborators for normal persons, except in Case 5, in which apparently none of the injected uric acid was eliminated The elimination of uric acid is subject to considerable individual variation The interpretation of the significance of total uric acid recovered following injection experiments is still a moot question For these reasons no stress is laid on the uric acid recoveries in this small series Indeed, Wells has made the point that urine recoveries of uric acid are valueless indexes of the fate of injected uric acid, since this metabolite may escape in unknown and unascertainable amounts through the intestinal wall

Regarding the rate of elimination of uric acid after the injection the chart shows that the maximum rate occurs from one to three and a half hours after the injection, and that at no later period does the rate exceed the initial peak There seems to be a gradual fall in the rate, and later increases probably are due to the ingestion of protein and carbohydrate foods

Thus it is concluded from the data obtained from these patients with eczema that they differ in no essential way from the results in normal persons recorded by other investigators

SCARLET FEVER IMMUNIZATION Use of Sodium Ricinoleate Toxin (Larson) in the Immunization Against Scarlet Fever Perkins R G and Megrall E Ohio State Med Jour, July, 1936 xxii 589

In a series of 1500 children of various ages 361 or 24 per cent were found susceptible by the Dick test using two test toxins checked against each other or known susceptibles and insusceptibles

Among the 66 susceptible adults, 8 or 29 per cent showed a negative Dick test three to six weeks after a single dose of 5000 units of Larson toxin

Among the 324 children available for retesting 210 or 64 per cent gave a negative Dick test two to five weeks after 3000 units of Larson toxin, while 62 others showed faint or doubtful reactions

If a negative Dick test is an indication of protection against scarlet fever, and since this reaction is very widely accepted as prima facie evidence it appears that this small dosage will protect some 64 per cent or more in a brief period

Only one dose was used in this series in order to determine this point but after retests this fall in the same series positives will be recommended to take at least two doses in the manner suggested by Larson Another series is in progress in which the positives have received two doses and will be retested this fall

Adults were much more resistant to immunization than were children The retests this fall will be followed by advice to the positives to take two or three doses as above

The very small series of contacts in whom Larson toxin was inoculated as a prophylactic is of course without weight as itself and valuable only as checking with the larger series of Larson

If the immunity is shown to be of long duration the absence of serious reaction and the lack of necessity for refrigeration of the toxin offer marked improvements in our activities against scarlet fever

ARTHRITIS The Classification and Treatment of Chronic Arthritis Cecil R L and Archer B H Jour Am Med Assn Sept 4 1926 lxxvii 741

In a study of 612 cases of chronic arthritis approximately two thirds (66 per cent) were of the proliferative type and one third (30 per cent) were of the degenerative type Proliferative arthritis occurs most frequently in young people, degenerative arthritis is more often seen in the middle aged and elderly

The commonest form of proliferative arthritis is associated with focal infection about the teeth or tonsils

The commonest variety of degenerative arthritis is the arthritis of the menopausal

The proliferative type of chronic arthritis is presumably an infectious process Degenerative arthritis has not been proved to be of an infectious nature It appears more likely that this form of arthritis, as the name implies, is a degenerative process analogous to arteriosclerosis and the other degenerative changes that attack various organs in middle age

The essential feature in the treatment of proliferative arthritis is the removal of all foci of infection In degenerative arthritis the treatment should be directed chiefly toward accelerating metabolism with iodides physiotherapy and other appropriate measures Many of these patients are improved by a low caloric diet

B ACIDOPHILUS AND B BULGARICUS Viability of B Acidophilus and B Bulgaricus in the Human Intestine Kulp W L Jour Am Med Assn Sept 11 1936 lxxxviii, 833

A report of studies from which it is concluded that B Bulgaricus will not develop in or survive passage through the human intestine, and that, therefore such preparations are without therapeutic value

PNEUMONIA The Epidemiology of Pneumonia, Powell, J P, Atwater, R M, and Felton D D *Am Jour Hyg*, July, 1926, vi, No 4, p 370

The purpose of this investigation has been to study the distribution of pneumococci among normal persons, the persistence of the organism in the respiratory tract, the virulence of the organism, and the existing relationships between the carrier state and season, age, sex and occupation

This study covers 418 observations on 93 persons in four groups—high school boys, medical students, student nurses and laboratory workers—all living in the same city and during the same period

There is good evidence that very few persons can long escape contact with some form of the pneumococcus under the conditions of our ordinary life. Moreover, contact with one of the fixed types of pneumococcus, forms associated with 80 per cent of all cases of pneumonia, is demonstrated for half the group during a period of seven months. Presumably, then, practically everyone at some time during the course of a year is a carrier of a fixed type pneumococcus

Waves of unusual prevalence of the fixed types of pneumococci are described. These were not accompanied by corresponding cases of pneumonia in the same group. The figures for that year, in Boston, indicate that there was about one case of pneumonia for every 175 residents during the period of investigation. This group studied consisted of 93 persons, so that the fact that only one person developed the disease was within expected limits

The observed prevalence of carriers of the different types of pneumococci, charted by months, suggests that we are dealing with organisms that go through seasonal variations in prevalence, variations which are probably different for the several types of the pneumococcus. The study of virulence by months also suggests that we are dealing with a biologic function of the parasite, analogous to well known seasonal activities in the plant kingdom

Study of the persistence of pneumococci in the respiratory tract of carriers shows that at least the fixed type organisms tend to disappear from the mouth and throat in less than thirty days. This was true in 37 instances out of 45 carriers who were found with these organisms. There was one instance in which a Type III pneumococcus was found in a person over a period of at least one hundred and twenty days, and there is reason, from virulence studies, to believe that this was persistence rather than reinfection. Three other cases showed persistence of from sixty to ninety days, but these were decidedly the exceptions. Type IV pneumococcus shows a persistence which is regarded as apparent rather than real, because of the variability in virulence of successively recovered strains and also because of the multiple opportunities for reinfection with indistinguishable strains of this type

There appears to be no significant difference between the sexes in their tendency to become carriers of the pneumococcus. The four groups of persons show evidence that their mutual associations favor local contact and their pneumococci tend toward a uniform distribution of types within each group at one time

When normal persons carry fixed types of pneumococci, they are rarely able to give any clue as to the source of these pathogenic varieties, a fact that has been brought out before in studies from this laboratory. These observations confirm the fact that persons in contact with cases of pneumonia more frequently carry fixed type pneumococci in their throats than controls, but carriers of fixed types are rather infrequent among the population at large. Persons may also get their pneumococci from contact with carriers. Therefore the isolation of cases of pneumonia could not be expected to be more than partially effective in controlling the disease

Studies on the virulence of 216 strains isolated during this investigation and tested on white mice, indicate that Types I and III tend toward a higher level of virulence than Types II or IV. In comparison with another study made in Boston and during the same year, there is observed an essential consistency of the virulence in all types recorded excepting Type II, in which an unusual number of virulent strains was encountered

The findings in this study, when compared with similar material from other sources, shows an essential consistency. In this composite fashion the data on carriers of pneumococci among 895 normal persons are given by types. Another comparison of data from 868

persons in Boston, all of whom were examined in this laboratory shows the definite excess of carriers among normal persons exposed to cases of pneumonia

In general these data serve to confirm the view that the disease pneumonia appears when there is a coincidence between infection with a pathogenic form of the pneumococcus and a lack of resistance in the same individual

MEASLES *Skin Tests in Measles* Tunncliffe E and Taylor E E Jour Am Med Assn, Sept 11 1926 XXXIII, 846

The green producing diplococcus associated with measles produces an extracellular toxin which gives a definite specific skin reaction in persons with a negative history of measles, but not in those with a positive history. The toxin is neutralized by coagulescent human measles serum and by the serum of goats immunized with measles diplococci but not by normal goat serum

These experiments indicate that antigens of killed measles diplococci (intracellular toxins) would be more effective for susceptibility tests than extracellular toxins on account of the former giving positive skin tests in about twice as many persons susceptible to measles as intracellular toxins (filtrates)

SYPHILIS *Syphilis of the Placenta in the Negro* McCord J R Ann Jour Obst and Gynec, June 1926, XI 6

Syphilis was demonstrated in 15.4 per cent of the 1000 placentas examined

In 966 women the Wassermann was positive in 22.5 per cent

In 655 babies, the cord Wassermann was positive in 0.7 per cent

The Wassermann reaction repeated on 396 women agreed in 88 per cent

Syphilitic placentas were found in 40.6 per cent of the positive Wassermann cases and in 0.3 per cent of the negative Wassermann cases

In 84 premature babies born alive the placentas were positive in 28.5 per cent

In 84 premature babies born dead the placentas were positive in 67.8 per cent

Of 219 positive maternal Wassermans the cord Wassermann was positive in 14.5 per cent of the cases

Of 747 negative maternal Wassermans the cord Wassermann was positive in 0.107 per cent

Of the positive placentas 75 per cent had positive maternal Wassermans

FOREIGN BODIES IN THE LUNG *Pathologic Changes in Lung Tissue as the Result of Foreign Bodies of Long Sojourn* Manges W F Jour Am Med Assn September 25 1926, LXXXIII 987

For the purpose of this paper a foreign body is one of long sojourn after it has been present in the air passages for two months or more and up to as many as thirty five years except that in one or two instances the sojourn has been less than two months but the pathologic changes are unusual. Serious pathologic changes do occur at times in cases of much shorter duration but these are more or less constantly the acute type such as infection emphysema or atelectasis with which physicians are quite familiar

An aspirated foreign body in any portion of the tracheobronchial tree will sooner or later cause extensive permanent injury

There is great variation as to the length of time a foreign body may be present before causing extensive changes, but those that interfere with drainage do, as a rule cause injury early

The permanent pathologic change is distal to the point at which drainage is blocked. The foreign body is at or distal to this point

The end results are in the nature of atelectasis fibrosis bronchiectasis and chronic abscess with varying quantities of purulent exudate. Hemorrhage is common tuberculosis is often suspected but is rarely present. The other lung remains remarkably free from pathologic change

Manges believes that many of the one sided, chronic, basal infections are the result of foreign body, regardless of history or of roentgenographic shadow of foreign body. Such lesions should at least be investigated bronchoscopically, and many should be treated in this manner.

FOREIGN BODIES IN AIR PASSAGES Live Fishes Impacted in Food and Air Passages of Man, Gudger, E W Arch Path and Lab Med, September, 1926, 11, 355

Gudger, who is bibliographer to the American Museum of Natural History, has collected from the literature all the reports of impaction of live fishes in the human throat and air passages.

The paper is most readable and of extreme interest but does not lend itself to abstraction.

LABORATORY TECHNIC

KOTTMAN REACTION The Kottman Reaction in Dysthyroid, Neuropathic and Psychopathic Children, Mattel, G Clin Ped, May, 1926, viii, 28

The author tested the Kottman reaction on seventy children with endocrine disturbances and various neuroses and psychoses and found that it showed great variability with out any special uniformity in dysthyroid children while the reaction was almost constantly slight in idiots, cerebroplegics and epileptics (who had been given iodine treatment), this latter finding agrees with the original findings of Kottman.

As to the genesis of the reaction the author believes that the degree of viscosity of the blood plays an important part in it independently of the effect on the viscosity of changes in thyroid metabolism.

LYMPHATIC LEUCEMIA The Neoplastic Nature of Lymphatic Leucemia and Its Relation to Lymphosarcoma, Evans, W A, and Leucutia, T Am Jour Roent and Radium Therapy, June, 1926, xv, No 6, p 497

Three cases of lymphosarcoma are described which in their terminal stages changed into lymphatic leucemia. These three cases are the only lymphosarcomata in a group of sixteen patients treated by deep roentgen ray therapy during the last four years, which had a fatal termination. In all three cases radiation therapy produced prolongation of life with entire disappearance of the manifest lesions treated, but later extension to the bone marrow occurred. At the time of the bone marrow involvement, all three patients had developed the picture of lymphatic leucemia. Evidence is thus brought that lymphosarcoma, if life is sufficiently prolonged, may change into lymphatic leucemia. This happens as soon as the bone marrow involvement becomes the predominating feature of the disease.

The paper is accompanied by eighteen illustrations.

TYPHOID FEVER Typhoid Vaccination by Mouth, Burke, V, and Barnes, La V Jour Infect Dis, July, 1926, xlix, No 1, p 67

The work of several investigators indicates that certain bacteria introduced into the intestinal tract have antigenic action. Evidence of the antigenic action consists of positive agglutinin tests and increased resistance to infection. Evidence was obtained from both animal and human subjects.

The experiments described in this paper were designed to determine whether typhoid proteins were absorbed unaltered from the digestive tract as determined by the appearance of agglutinins, whether the agglutination titer would vary with the method of preparing the vaccine, whether "per os" vaccination is as effective as the subcutaneous method in stimulating the production of typhoid agglutinins, and to test the theory of increased cellular impermeability of the intestinal mucosa following the oral administration of typhoid vaccine.

The experiments were conducted on rabbits the conclusions reached being as follows
Agglutinins appear in the blood of some rabbits following introduction of standard typhoid vaccine by mouth. Artificial erosion of the intestinal mucosa in such rabbits is unnecessary.

Subcutaneous vaccination is more effective than vaccination by mouth in stimulating the production of typhoid agglutinins in rabbits.

The comparative degree of immunity to typhoid conferred by the two methods of vaccination remains to be determined. The comparative rate at which immunity develops considered separately from the appearance of agglutinins is also unknown. Since subcutaneous vaccination affords adequate protection against typhoid any superiority of the oral method considered from the practical standpoint is limited to simplicity of administration and reduction in toxicity.

The absorption of typhoid protein from the intestinal tract of rabbits apparently decreases upon continued exposure of the mucosa of the protein.

RHEUMATIC FEVER Significance of the Leucocyte Count as an Index of Rheumatic Infection in Children Wilson M G and Kopel M. *Am Jour Dis Child*, July 1926 xxvii 46

From a study of 384 counts on 65 children ranging in age from four to fifteen years the conclusions following were drawn.

The average leucocyte count for children with a history of previous rheumatic infection (potential heart disease) was 7000 cells.

The average leucocyte count for children with organic heart disease (Class 1) was 7700.

The average leucocyte counts for children with active rheumatic infection (carditis arthritis, chorea and recurrent growing and joint pains) were above 9000 cells.

The correlation between vital capacity determination and leucocyte counts in children with rheumatic heart disease confirms the modern conception of cardiac failure in children being primarily due to infection rather than to mechanical factors.

Leucocyte counts within the normal range may be considered as indicative of the quiescence of the rheumatic infection and counts above the normal range indicative of activity of the infection.

Repeated leucocyte counts in children with rheumatic infection would seem to be of some clinical value in diagnosis prognosis and treatment.

PERNICIOUS ANEMIA The Diagnostic Value of the Color of the Blood Serum in Pernicious Anemia Fishberg M. *Am Jour Med Sc* July 1926 clxxv No 1 p 81

Fishberg notes that the color of the blood serum in pernicious anemia is always above normal and believes that this accounts for the characteristic color of the skin in this disease.

The serum pigmentation is generally due to an increase in the hibrubin content of the serum but not always and may sometimes be due to hematin.

Fishberg concludes that

In the active periods of pernicious anemia the blood serum is more highly pigmented than normally, being golden brown or rarely even dark brown. This is due to an increase in the hibrubin content and to the presence of hematin.

In an anemic patient the presence of pale blood serum speaks definitely against the diagnosis of pernicious anemia. Observation of the serum color is of particular value in the differential diagnosis between secondary anemia due to carcinoma and pernicious anemia.

MENINGITIS Meningitis Caused by Bacilli of the Colon Group Neal J B. *Am Jour Med Sc* November 1926 clxxv No 5 p 730

Meningitis due to members of the colon group is comparatively rare. In studying over 1500 cases of meningitis only 7 were found due to these organisms. In an eighth case there were several organisms present, one of which belonged to this group.

Meningitis in the first three months of life is most often caused by the meningococcus—³/₄ out of 50 cases being due to this organism and only 3 to the Bacillus coli.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughn, Medical Arts Building,
Richmond, Va

Radiotherapy¹

THIS little book is in no sense a reference book for the general practitioner, neither is it a short textbook. It is a general review on very broad lines of the subject of radiotherapy and its relation to the numerous branches of medicine. The fundamental principles of therapy are very well dealt with, and the effect of radiation on normal cells and diseased cells is well brought out. The attitude of the writer toward a single remedial measure is best explained by the closing paragraph of the book.

"Let us remember that nature recognizes neither the surgeon nor the radiologist, nor the expert in drugs, nor yet the lord of germs. She will put forth her best efforts only when she is assisted without stint. General practice is in reality the only logical form of practice. But no human mind can compass the whole field of medicine. Hence the hope of the future lies in specialism tempered by cooperation."

The Significance of the Physical Constitution in Mental Disease†

MEDICINE monographs are comprehensive reviews that adequately discuss a disease, certain aspects of a disease, or subjects that allow a better comprehension of disease processes.

In this, the tenth of this series of monographs, the authors have added an important contribution to the study of the relation of physical habitus to the reaction to mental disease.

In the early days of medical practice the family doctor was often credited with "knowing the constitution" of his patient and the trend of modern medicine which the reaction of the individual has led to interesting studies, as witness the contributions of Draper on the human constitution, of Dunn and Seegal on the correlation of facial form and disease and so on.

Wertheimer and Hesketh have undertaken an examination of mental patients from the standpoint of external habitus with the idea of testing the hypothesis that physical habitus and mental reaction types exhibit a more or less definite correlation. In these studies both simple observation and exact measurement (anthropometry), are utilized and, as a result of the observations thus made a new anthropometric index is proposed.

Beginning with a review of early studies of body types, and of anthropometric studies in disease and psychiatry, the authors then compare the classifications which have been proposed in the past and discuss in general the methods used.

Their own studies were conducted on 65 male patients chosen at random who are classified into four groups:

- 1 Predominantly affective reaction types
- 2 Predominantly schizophrenic reaction types
- 3 Organic reaction types
- 4 Psychopathic personalities and psychoneuroses

*Radiotherapy in Relation to General Medicine. By Francis Hernaman-Johnson. Radiologist to the French Hospital London. Cloth Pp 211. Oxford University Press.

†The Significance of the Physical Constitution in Mental Disease. By F I Wertheimer Associate in Psychiatry Johns Hopkins Hospital and F E Hesketh Carlton Fellow in Medicine Johns Hopkins University with a preface by L F Barker. Cloth Pp 76. 20 figures 5 plates. Price \$2.50. Williams & Wilkins Co. Baltimore.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Data were assembled from observation and psychiatric and anthropometric studies from the analysis of which the authors suggest a new anthropometric index based on the formula

$$\frac{\text{Leg length} \times 10^3}{\text{Transverse chest diameter} \times \text{sagittal chest diameter} \times \text{trunk height}} \times 100 = \text{index}$$

The remainder of the book discusses the application of the index to the types studied. The contribution is of distinct interest to students of internal medicine and psychiatry.

*Collected Papers from the Henry Ford Hospital**

IN THIS volume, as the title indicates are collected the scientific writings of the Staff of the Henry Ford Hospital from the date of its founding in 1915 until 1925. Although the period covered is of ten years' duration most of the papers were written during the past two years.

After a foreword there follows an alphabetical list of former staff members, a list of the present staff members and then the three sections of the book proper: forty-three papers on varied subjects; in Appendix A concerned with technical appliances and the concluding section Appendix B which is a concise account of the history of the hospital and of its physical equipment.

The papers are arranged in alphabetical order according to their authors. An arrangement or grouping in accordance with their subjects—surgical, medical, laboratory, etc.—would seem preferable.

The subject matter is divided as follows: papers concerned with the investigation and clinical application of blood chemistry 7; internal medicine 5; surgery 12; roentgenology 3; obstetrics 6; laboratory investigations 10; internal medicine, 5.

The volume is an excellent example of the printer's art and is of interest both to those interested in the hospital and as a source of convenient reference on the subjects covered.

Klinisches Lehrbuch der Inkretologie und Inkretotherapie†

THIS is a four hundred page book which attempts to give a cross section of the subject of the internal secretions in sufficient detail for the man doing clinical work. One third of the book is taken up with the structure and function of the glands of internal secretion; then follows a chapter on diagnosis and one giving rather complete clinical pictures of typical cases. Finally, there are chapters on the significance of the internal secretions in the various fields of medicine and these chapters are written by various authors: that on internal medicine is by von den Velden; that on psychiatry and neurology by Rosenfeld; pediatrics by Schiff; gynecology and obstetrics by Aschner; urology by Bachrach; ophthalmology by Szily and Poos; otolaryngology by Kobrak; and dermatology by Rothman.

There are short bibliographies at the end of each chapter but as is so common in German books since the war, the references to American literature are most noticeable by their absence. Abel is mentioned in the text in connection with the active principle of the hypophysis; Aldrich with adrenalin; Banting and Best with insulin; and Kendall with thyroxin, but in no case is there a reference to the time or place of their publications.

However the book gives in compact form the German point of view and so helpfully supplements some of our American texts.

Collected Papers by the Staff of the Henry Ford Hospital (First Series 1915-1925). Cloth. Pp. 534. 151 illustrations, 4 charts. Price \$5.00. Paul B. Hoeber Inc.

†Klinisches Lehrbuch der Inkretologie und Inkretotherapie. By Dr. Gustav Bayer and Dr. R. von den Velden. Georg Thieme 19.

*Preventive Medicine and Hygiene**

THE predominant function of the modern physician is the prevention of disease, and for an understanding of the principles underlying and applied to disease prevention a working knowledge of many and varied subjects is required. In this book, practically the only one of its kind, Rosenau has collected and presented in a most practical and utilizable manner, a vast amount of information of value to practicing physician as well as to the epidemiologist or the sanitary engineer.

This, the fifth edition of this well known work, has been largely rewritten and reset and has undergone extensive additions and revisions. The book in general has two main divisions: (a) dealing with personal hygiene, and (b) dealing with environment (sanitation).

The material presented in the sixteen chapters of this book constitutes a veritable encyclopedia of methods applicable to the prevention of disease evaluated by an authority of extensive experience and presented in a most interesting and clear cut manner.

New subjects which are considered comprehensively for the first time in this edition embrace the psychoanalytic approach to sex hygiene, the prevention of cancer, the conservation of vision, periodic physical examinations, flukes and their relation to disease, granuloma inguinale, balantidial dysentery, resuscitation, gas masks, statistical methods, and practical points in public health administration. For some of these subjects Dr. Rosenau has enlisted the help of expert authorities.

The section on granuloma inguinale consists of but two short paragraphs which, in view of the increasing numbers of cases of this disease which are being reported, seems rather brief. The sections on statistical methods should enable many to grasp such methods to whom they are now in an uncharted maze. Not only are all the important methods used in public health laboratories fully described, but numerous clinical procedures are also clearly detailed. There is practically no one, no matter how remotely interested in disease, for whom this volume will not prove an invaluable desk companion. It should be purchased for constant and daily use. It can be recommended without reserve.

The Inflammatory and Toxic Diseases of Bone†

AS STATED by the author, it is not easy to draw a line between the inflammatory and the toxic diseases as in both there are associated inflammatory changes, but a distinction may be made, however, between toxins locally produced by bacteria and circulating toxins from a distant source. The resulting conditions also differ, and the title of this book serves to indicate the distinction and the difference.

There are twenty chapters in which are discussed various bone lesions or diseases, namely, acute osteomyelitis, subacute osteomyelitis, serous periostitis and osteomyelitis, tuberculous disease of bone, syphilitic disease of bone, Charcot's joints, syringomyelia, jaws, arthritis deformans, pulmonary osteoarthropathy and allied conditions, rickets, infantile scurvy, osteitis fibrosa, osteomalacia, osteitis deformans, leontiasis ossea, and osteogenesis imperfecta.

Practically all of the illustrations are photomicrographs of gross or microscopic sections. These are excellently well done and greatly add to the value of the book.

The book amply reflects the experience of the author and is clearly and well written. There are numerous illustrative case reports some of which, however, are so brief and bald as to add but little to the text.

The author has drawn freely, and with profit, upon the collections of various English museums with the result that for each subject touched upon there are either gross or microscopic examples illustrated.

While not intended to be an encyclopedic discussion of the subject and, to some extent,

*Preventive Medicine and Hygiene. By M. J. Rosenau, Professor of Preventive Medicine and Hygiene, Harvard Medical School. Cloth. Pp. 1453 with 157 illustrations. Fifth edition. D. Appleton & Co., New York.

†The Inflammatory and Toxic Diseases of Bone. By R. Lawford Knaggs, Consulting Surgeon to the Leeds General Infirmary, etc. Cloth. Pp. 116 with 197 illustrations. William Wood and Co., New York.

reflecting the personal views of the author, the book well deserves a place upon the shelves of the student, the physician, the surgeon, and the pathologist as a valuable source of reference

The physical craftsmanship of the publishers is most excellent

*X Ray Therapy**

THIS little book of x ray therapy consists of 120 pages. It sets forth the method of treatment as practiced in Professor Holzkecht's Clinic in Vienna. A short section deals very closely with general considerations of treatment. It is well worth reading even by those not practicing x ray therapy.

The greater part of the book deals with treatment of about seventy five various diseases. Most of these are conditions in which the x ray has proved of definite value. A very few are still in the experimental stage. Each disease is considered in the following manner:

- Prognosis—
 - a Result of treatment
 - b Duration of treatment
 - c Course of disease under influence of x ray
- Accompanying and subsequent effects—
 - Adjuvant treatment
 - Contraindications
 - Treatment formula
 - Fields

The treatment formula is clearly set down. It is given in Holzkecht's units of course.

The book is cleverly compiled and makes x ray therapy seem quite easy. It is intended as a guide for the man in general practice in deciding which cases are amenable to treatment, and a book of formulas for the radiologist. It serves both purposes well, but perhaps not as thoroughly as if they were dealt with separately.

Bacteriologic Atlas†

THOSE interested in bacteriology will recall the publication twenty five years ago of the *Atlas of Bacteriology* by Lehmann and Neumann of which the atlas here reviewed is reminiscent.

An illustration which illustrates is more rapidly, and sometimes more definitely, in formative than several descriptive paragraphs, whose interpretation depends to no small extent upon the imaginative acuity of the reader.

The purpose of this atlas is to convey a picture of the various microorganisms illustrated as seen with the microscope under a magnification of about 1000 to 1500 diameters. The illustrations are drawn from tissue sections and from film stained preparations and are accompanied by a brief description of the main features illustrated.

There are sixty colored plates, beautifully prepared and beautifully reproduced, all representing actual specimens from the author's collection. In a few instances (amebae, malarial plasmodia) forms from various parts of the slides are represented as appearing in one field.

For teaching purposes this should prove a very useful and valuable little book.

The illustrations are very well done and the color reproductions are remarkably faithful.

Index and Handbook of X Ray Therapy. By Dr. R. Lenk, Privat Dozent of Medical Roentgenology, University of Vienna, with a foreword by Professor Holzkecht. Translated by T. I. Candu, Hon. Radiologist, Royal Ghent Hospital. Cloth. Pp. 170. Oxford University Press.

†Bacteriologic Atlas. By Richard Muir, Demonstrator of Pathologic Methods, University of Edinburgh. Cloth. Pp. 134. Price \$4.50. Wm. Wood & Co. New York.

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Official Organ of the American Society of Clinical Pathologists

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EDITORIALS

Histories

THE foundation of many a reputation was laid when its possessor was an interne taking histories

To many residents, history taking is a bugbear and a nuisance, often because of failure to realize that the hospital history is a deluxe edition of the stories to be elicited later in the office

Every patient has a story to tell, sometimes without knowing it, and the development of the tale in its entirety very often depends almost solely upon the ability of the interrogator. As has been said, he must combine with the shrewdness and pertinacity of a prosecuting attorney, the finesse and tact of a diplomat

Not infrequently a great mass of more or less irrelevant and nonessential details must be elicited and sifted to obtain the kernel of the story which may then be expressed in a sentence

The taking of a good history is an art and a revelation, at the same time, of the skill and general knowledge of its compiler

Each patient, in effect, subjects his physician to a general and searching examination as to his knowledge of the entire field of medicine. Very few patients will be prepared to recite a classical textbook description of their complaint.

It is not only what they reveal but what they wilfully or unwittingly conceal which is often of paramount importance and which must be elicited by devious and tactful methods.

Pathognomonic symptoms are the exception. Most often it is suggestive "leads" unexpectedly discovered or suspected and unearthed which when elaborated and dovetailed with the other data lead to the ultimate clearing up of obscure conditions.

No part of the body is functionally a distinct entity from all the other parts and detection of a cardiac lesion or a renal deficiency should not be the termination of the examination but rather the starting point of an endeavor to correlate this finding with the rest of the human machine.

Thoroughness first, last and all the time is the keystone of success.

Histories have other uses besides their application to the present case. Every good physician is a continual student and the mine of his past experience often holds nuggets not to be found in books. Well taken and carefully kept histories more than repay an occasional rereading and study and bring to future cases the experience and information gained from past studies.

Moreover, the development of medicine depends upon the study and elucidation of past experience and it is indirectly, from the compiled professional memoirs of physicians at large that the textbooks of the future will be written.

It is not enough to record good histories; they must be studied continually and utilized. Carefully taken intelligently filed so as to be readily accessible, they form a permanent record of past experience and an accumulation of knowledge for future use.

Histories are compilations, condensations and accumulations of answers to questions put by the examiner and the *meat of the answers may often be expressed in a sentence*. The value and significance of that sentence in suggesting a possible diagnosis depends as much upon the questions put as upon the answers received.

It is sometimes of little avail to ask a patient point blank whether he has had a venereal disease or is a heavy drinker, for many patients are very Chinese in their endeavors, for reasons hard to fathom to "save their face" and still others are supplying misinformation honestly.

An old Scotch doctor in charge of a dispensary in a section where the morning emesis and nausea of preprohibition days was comparatively common, was never known to ask the bulbous nosed sufferer if he was a heavy drinker. He merely suggested that relief was sometimes obtained by a small drink of whiskey in the morning before breakfast of course the *first* one might not stay down, but the second would and gave comfort.

Who could refuse in the presence of such understanding to relate how the remedy was well known and often tried with the described result? Then

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CLINICAL AND EXPERIMENTAL

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

THE RELATION OF CLINICAL PATHOLOGY TO PRECLINICAL MEDICINE*

By DR WM C EATON NEWARK N J

MANY years ago when I was doing general practice I had to listen so often and so long to what folks had to say about their babies and their children that I vowed that if ever I had any I would never tell about mine. Well when I got older I did have some children but as mine were really just a little bit different from other folks I was never quite able to keep that vow.

Again it has been my good fortune to enjoy many a dinner with plenty of company around me only to have the effect somewhat neutralized by a so called "presidential address." Here too I made resolutions and likewise find it impossible to keep them as perfectly as I want.

As I get older I find myself more and more agreeing with Phillips Brooks who once defined life as a series of compromises and if you will permit it, I will compromise with you by not insisting on you a real presidential address but rather running through a few thoughts which have occurred to me from time to time in connection with the latest development in the practice of medicine which is sometimes spoken of as "health examinations" but which I prefer to designate by some such term as "preclinical medicine." I think this movement is a most important one which had to wait until medical science had advanced to a certain point before it was worth while putting into practice although the idea is not at all new.

One of our honored guests has asked me to define "clinical pathologist." May I say that to my mind clinical pathology represents an array of facts and procedures the offsprings of scientific research and that the clinical pathologist is a specialist who bridges two gulfs one between the research laboratory and the practitioner, and one between the physician or surgeon and the patient. New facts and methods are constantly being brought to

* Presidential address at the Sixty-first Meeting of the American Society of Clinical Pathologists Washington D C May 14

when the patient had gone, with a quizzical look through his spectacles, the diagnosis would come "alcoholic gastritis"

One must be on the alert in taking histories for unconscious hints as to further interrogations. Remember Cabot's simile of the cash register and devise your questions accordingly.

Investigation, correlation, and interpretation are the keystones of diagnosis

—R A K

Immunization Against Tuberculosis

THE hope of an efficient vaccination for tuberculosis has been revived in recent years by Calmette, who advocates the use of an attenuated organism of bovine origin, *Bacillus Calmette Guérin* (BCG)

The *Beitrag zur Klinik der Tuberkulose*, volume LXVII, contains an interesting symposium on artificial immunization against tuberculosis. Uhlenhuth calls attention to the need of finding out how nature at times succeeds in bringing about immunity to the tubercle bacillus, and recalls that immunity only exists when a focus of tubercle is present. The relative immunity of individuals depends on a more or less latent infection of childhood. The lowering of the vitality of the body, such as occurred in Germany during the year of hunger of the World War, allowed a spread of disease from latent foci.

The exact nature of immunity from focal infection is not known. Should a focus of tubercle completely heal, immunity is lost. Uhlenhuth relates experiments in immunization of cattle by injection of an old bovine culture isolated by v. Behring in 1902 from the mediastinal gland of a cow. The culture was only moderately virulent for guinea pigs, avirulent for rabbits, and almost entirely avirulent for cattle. The results of these experiments as tested by artificial and natural infection were completely negative. In view of such findings Uhlenhuth expresses surprise that Calmette with a somewhat similar culture should have succeeded in immunizing cattle. He criticizes Calmette's experiments in that droplet infection plays the principal rôle in producing disease in cattle, and he believes the experimental cattle of Calmette were protected from this by their positions in the stalls. Evidence is produced from other workers which indicates that the BCG of Calmette can produce tubercles and that these may even at times go on to caseation. He agrees, however, that BCG is practically harmless and that years would be necessary to show that it might regain its virulence in the human or animal body. Avirulent cultures are useless for immunization purposes, as shown by Romer, Neufeld, Kraus, Selter and others, and Uhlenhuth states that if Calmette's work is proved, we are in contact with an absolutely new fact in immunization against tuberculosis.

—G B W

clusion regarding clinical pathology as Dr. MacEachern found in the clinical field. That conclusion was arrived at after studying thousands of examinations of apparently healthy people made by expert medical examiners and has since been substantiated by experience and by every bit of evidence that it has been possible to gather.

If you read any of the articles which have found their way into different journals, it makes no difference from what part of the country they have emanated, or whether they have been published by clinic or practitioner or some other kind of agency interested in health and if you will examine the available statistics, you will see that in the preclinical field it almost always requires some kind of a laboratory examination to throw the necessary light on the great majority of cases which need medical help. Our study of an adequate human material has shown conclusively that the number of people who are healthy enough not to have to complain of symptoms or discomforts but who show physical signs on examinations are relatively rare as compared with the unexpectedly large number who are found to have significant health impairments by laboratory tests. Furthermore the laboratory detects much more often than the physical examination changes which can be correlated with presymptoms, the evidences of slight malfunction or beginning disease. I was particularly glad that in our program for this meeting there were two papers on the subject of clinical pathology and dentistry, a very important subject in the preclinical field which is still in need of a great deal of constructive work.

The subject I have chosen is a very large one but I am not going to attempt to cover it. I do however want to mention something that from time to time, one sees in the medical journals. I am speaking of the activities of the life insurance companies and their interest in the promotion of longevity. Many of the articles show much confusion of thought for instance in such a slogan as "no middleman is needed in practice." From reading them one gathers that there is a more or less widespread idea among physicians that life insurance companies have a kind of interest in the matter of preclinical medicine which is in its nature socialistic or tending to socialize medicine. I can speak with authority and know for a certainty, and believe that if you will think for a moment about it, you will not fail to understand that nothing can be further from the truth. In the first place it is inconceivable that insurance companies will ever for a moment think of practicing medicine or having anything to do with the treatment of sick people. The surpluses which they have the responsibility of accumulating for the benefit of policyholders offer too easy a target for unscrupulous or dissatisfied patients, and because there can be no practice of medicine without treatment, the intervention of insurance companies in the practice of medicine is an impossible absurdity. All that a life insurance company can ever hope to do in conserving the health of its policyholders is to try to do whatever it can to help policyholders get the best possible scientific medical treatment instead of falling into the clutches of quacks, cults, and other fakers. Out of my own experience I can say with certainty that the active participation of life insurance companies in promoting longevity results not

light which offer the physician and surgeon a truer insight into the diagnosis and treatment of disease, and the practitioner who does not use clinical pathology well gives his patient unscientific and inferior medical service

I hope you will let this pass for a definition of clinical pathologist, because when it comes to defining preclinical medicine, I find there is considerable confusion and that the literature of the subject is chiefly remarkable for what might be called "glittering generalities." In what has been written, there seems to be no distinction made between people who have no health complaints at all and people who have what Dr. Gould called "presymptoms." Obviously people who feel perfectly well are the only ones who can profit by periodic examinations. Those who have presymptoms like overweight, underweight, frequent colds, indigestion, rheumatic tendencies or tendency to shortness of breath, nervousness, and what have been hitherto generally considered negligible symptoms are not in need of routine or periodical examinations. They need treatment based on adequate diagnosis. I think it would improve matters to limit the periodic examinations to people who have no health complaints at all and to invent some other slogan such as "See your doctor early as no symptom is trivial enough to neglect," for people who have complaints and symptoms, no matter how slight they are, especially if there be any tendency for them to persist. With this distinction preclinical medicine would then be limited to those who have early or presymptoms.

Now the connotation involved in the concept "presymptom" is that borderline field between health and disease which good physicians are constantly struggling to narrow by making the diagnosis earlier and earlier. It is the most difficult of all fields of medical practice because the earliest evidence of malfunction is usually a symptom which has heretofore usually been regarded as negligible, and which we here call the presymptom. It is not so picturesque or impressive as the frank symptom of disease and therefore much more difficult to interpret and diagnose. Men who have devoted their lives to insurance work, however, are quite convinced that even the slightest of persistent symptoms or the faintest evidences of malfunction or the most benign of vicious habits affect longevity. In other words, preclinical medicine interprets and relieves, instead of neglecting, what have hitherto been regarded as negligible symptoms. It means, as Mr. Little says, "giving an individual continuous care instead of merely spasmodic attention on a breakdown."

Now, I admit that when I investigated the whole matter of health conservation some years ago with the view of constructing a plan which in operation would promise to prove most serviceable in conserving the health of people representing the general population, I spent five or six years getting what light I could on the matter by gathering statistics, with the help of expert statisticians and mathematicians in evaluating them. As a result it was impossible to come to any other conclusion than that by and large the practice of medicine throughout these United States more than anything else needed more and better facilities for giving people who were not considered actually sick the advantages offered by clinical pathology. Of course our viewpoint extended only over the field of preclinical medicine, but you will note that the result of our investigation led us to about the same con-

ADDRESS*

By REV. S. J. MOULNIE

THIS was entirely unexpected on my part but your esteemed President urged me to say a few words. I would like to have the opportunity of speaking to you tonight at your banquet because there are a few things that I have in mind that you might like to hear not that there will be anything new to you but they might have a little note of encouragement in them.

I want to congratulate your body of men on what you have been doing and what I am sure you are going to do in the future for scientific medicine. The practicing profession of the usual hospital is only slowly as far as I am able to observe becoming imbued with the final findings of the laboratory as signs in diagnosis and in treatment. All of you know this. I cannot tell you anything new. I would like to encourage you to develop the experience that you must have in a gathering of this kind and get it out into the field. I understand there are three or four hundred first class pathologists. You all know how difficult it is for the hospitals to get men who are reliable, safe and sound clinical pathologists. You know, too, how difficult it is to get the staffs to have confidence in the pathologist. An organization like this it seems to me is bound to bring out a larger number drawing them from the medical schools into this work. It is going to make the importance of the pathologist more clearly appreciated by the profession and I think therefore in the next five ten fifteen years you will be what you should have been all the time a specialist in medicine consultants always in the hospital and relied upon and looked up to by the whole profession. I want to see the pathologist where he wants to be. There is hope of salvation for scientific medicine in the hospital. The College of Surgeons and our Association are working closely together trying to what we call standardize the hospital. It isn't the hospital that needs it so much, it is the medical profession. They need to realize more and more what scientific medicine is, and as far as possible be brought to practice it. Nearly every pathologist admits that ten years ago he was usually very discouraged about things. His laboratory wasn't being used as it should be. He couldn't get the men to appreciate it. He was seldom if ever taken into consultation. His work was looked upon as dispensable.

I hope you use your influence on the hospital phase of standardization and bring it about that the financial problem will not be so miserable as it has been in the past, that you will have better compensation so that you can attract better men into your specialty, so that your number will grow in some way commensurate with the great number of hospitals. There are 7000 hospitals and 400 acceptable pathologists. What is being done to meet the situation? One man will have three or four hospitals. The fact that they are using men in so many places is an indication that they are coming to appreciate it more and more.

Address to American Society of Clinical Pathologists Sixth Annual Convention Washington D. C. May 14 1927

only in discovering thousands of people in the early stages of disease at a time when they can be helped but also in finding large numbers of neglected people who have been discouraged or victimized by negligent and ignorant physicians, usually graduates of colleges happily no longer in existence. The cooperation of the life insurance companies in finding these people and trying to save them from the hands of fakery and ignorance would be regarded as a real accomplishment in the salvage of life if the truth were fully realized. From what I know about the attitude of life insurance companies, they constitute the staunchest bulwark that now exists against the introduction of anything socialistic into the practice of medicine, and every bit of evidence I have ever seen on the point conclusively shows this. Those of you who fear the menace of socialism in medicine should look in other directions and begin in our own ranks with the few traitorous physicians who compromise all of us by being greedy, incompetent, negligent or meddlesome. There are also other abuses, take the state laboratory for example which medical men are perpetuating into a real socialistic menace without realizing what they are doing. Certainly no time should be lost in making doctors understand that they are often unintentionally bringing about a drift toward socialistic tendencies. This drift, I feel convinced, does not happen as the result of any special design but simply as a consequence of incoordination between the different branches of the medical profession, especially those interested in public health activities.

Thus, only a few weeks ago the *Journal of the American Medical Association* carried a very good editorial. It seemed to me as if it had been written because some health officer complained of lack of cooperation between practitioners and health authorities. Of course the gist of it was that practitioners and the health authorities should cooperate. There is no other way about it, such cooperation is absolutely essential if we are to have a more complete and better understanding between the various branches of the medical profession. Once we have learned to coordinate our activities, a spontaneous cure of most of the difficulties which now occupy our minds will, I feel certain, be effected. Clinical pathology has suffered from lack of coordination and cooperation more than any other specialty of medicine and with the result that the public has not yet received its full measure of the advantages which adequate clinical pathology can yield. On the other hand, clinical pathology needs encouragement in order to advance scientifically more rapidly and to attract better and more men to it.

In closing, I desire not only to emphasize the value and necessity of educating medical practitioners regarding the advantages which clinical pathology affords them and their patients in the diagnosis and treatment of disease, but particularly to point out that clinical pathology also offers numerous possibilities for extending and improving the field of preclinical medicine, and that research along these lines can confidently be expected to produce important results in bettering the health of the nation.

five years of age, 7 or 39 per cent were between five and ten years of age and 8 or 44 per cent were between ten and fourteen years of age

From the above figures it seems that orris root sensitivity in allergic children is not uncommon. Two interesting case histories deserve brief discussion.

CASE 1—D W, male, age five years, has been a subject of perennial hay fever, eczema and asthma for three years. The hay fever is much more severe in September and October. The mother has noticed that he would usually develop a congested nose and lacrimating eyes on attending the theatre, and the symptoms would occasionally be associated with a slight attack of asthma.

Intradermal tests revealed the following:

Orris root	+++
Giant ragweed	+++
Short ragweed	+++
Western ragweed	+++

No other inhalant atopic substances were positive.

Treatment with an extract from orris root and one made up of the three ragweeds gave the boy complete freedom from symptoms.

CASE 2—M J H, female, age three years, has suffered from eczema and asthma since three weeks of age and perennial hay fever during the past year.

Intradermal tests showed the following reactions:

Eggs	+++
Wheat	+++
Cat hair	+++
Orris root	+++

Removing the foods to which she was specifically sensitive from the diet and the use of unscented cosmetics in the home gave her entire relief from symptoms.

In the first case it appears that orris root was the sole cause of his perennial hay fever and asthma, but the ragweed produced typical seasonal hay fever. In the second case orris root was only a contributing factor. Later in life this child will naturally be brought in contact with much greater amounts of orris root, and in all probability will have to be desensitized against it if she hopes to be free at that time from her asthma and hay fever.

Of 82 perennial adult hay fever cases, 39 or 47.5 per cent, reacted definitely to orris root. In 6, or 7.3 per cent, it was considered the sole factor. In 33, or 40.2 per cent, it appeared to be only a contributing factor. Any product that is either the sole cause or a contributing factor in 47.5 per cent of perennial hay fever deserves careful consideration. Two case histories relative to this condition will be briefly discussed.

CASE 3—E M C, female, age thirty-two years, has suffered from lacrimating eyes in a moderate degree for three years. She complained of slight itching of the inner canthus of the eye and also had a mild congestion of the mucous membrane of the nose at times with some itching of the nasal mucous membrane. She was advised by a competent eye specialist to be fitted with glasses although he told her he found no special eye defect. She soon learned that wearing glasses did not change the symptoms of which she complained. Two other oculists were consulted and each fitted her with a pair of glasses but without relief.

Intradermal testing showed a four plus reaction to orris root but a reaction to no other atopic substances.

The College of Surgeons is giving its attention to the proper equipment and proper personnel of the laboratory. They started out using the word adequate laboratory service. They are coming to define what that means, in equipment and routine tests. But what I consider the best of future value I have mentioned, namely, that the staffs more and more are coming to look upon the pathologist as a real consultant on an equal standard with themselves. I am particularly sure that you all want that. It is the only standing for you to have. I want to thank you very much for inviting me to say these few words. Whenever I can I am going to further the interests of the clinical pathologist in the hospitals. You should get after them in the schools and the younger members of the profession so that there will be more and more coming into existence.

We are getting more and more interested in technicians. We want to follow out your rules and regulations and requirements for their training.

We can naturally help one another, and I know you will give me encouragement and help in what I am trying to do.

THE IMPORTANCE OF ORRIS ROOT AS AN ETIOLOGIC FACTOR IN HAY FEVER AND ASTHMA*

BASED ON THE STUDY OF ONE THOUSAND CASES OF HAY FEVER AND ASTHMA

BY RAY M. BALYEA, M. A., M. D., OKLAHOMA CITY, OKLA.

SEASONAL hay fever has long commanded the intense interest of those working in the field of allergy. The interest is largely due to the peculiar and startling suddenness with which symptoms appear and the dramatic cessation of symptoms with the onset of frost. The relationship between pollen and seasonal hay fever is well established. There is another condition, however, which bears a train of symptoms similar to seasonal hay fever, but it is somewhat irregular in occurrence and has no relation to the month or season of the year. Recently careful attention has been given to the investigation of its cause. The terms rose cold, vasomotor rhinitis, hyperesthetic rhinitis, atopic coryza, perennial hay fever, and other names have been applied to the train of symptoms. Of these terms perennial hay fever is probably most descriptive. The causes of perennial hay fever are many, of which orris root is one of the most common. I wish to present some data relative to the importance of orris root as an etiologic factor in perennial hay fever, asthma, and other allergic diseases.

This work is based on the study of 1,000 cases of hay fever and asthma, of which 180 were hay fever and asthma cases in children, 82 were adult perennial hay fever cases, 365 adult asthma cases, and 373 were adult seasonal hay fever cases. They were all American born, private cases, ranging in age from four months to eighty-six years.

Of the hay fever and asthma cases under fourteen years of age 18, or 10 per cent, reacted to orris root. Of this number 3, or 15 per cent, were under

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five years of age, 7 or 39 per cent, were between five and ten years of age, and 8 or 44 per cent, were between ten and fourteen years of age

From the above figures it seems that orris root sensitivity in allergic children is not uncommon. Two interesting case histories deserve brief discussion

CASE 1—D W, male age five years has been a subject of perennial hay fever, eczema, and asthma for three years. The hay fever is much more severe in September and October. The mother has noticed that he would usually develop a congested nose and lacrinating eyes on attending the theatre and the symptoms would occasionally be associated with a light attack of asthma.

Intradermal tests revealed the following:

Orris root	++++
Grass ragweed	+++
Short ragweed	++++
Western ragweed	++++

No other inhalant atopic substances were positive

Treatment with an extract from orris root and one made up of the three ragweeds gave the boy complete freedom from symptoms

CASE 2—M J H, female age three years, has suffered from eczema and asthma since three weeks of age and perennial hay fever during the past year

Intradermal tests showed the following reactions:

Eggs	++++
Wheat	++++
Cat hair	+++
Orris root	++++

Removing the foods to which she was specifically sensitive from the diet and the use of unperfumed cosmetics in the home gave her entire relief from symptoms

In the first case it appears that orris root was the sole cause of his perennial hay fever and asthma but the ragweed produced typical seasonal hay fever. In the second case orris root was only a contributing factor. Later in life this child will naturally be brought in contact with much greater amounts of orris root, and in all probability will have to be desensitized against it if she hopes to be free at that time from her asthma and hay fever

Of 82 perennial adult hay fever cases 39 or 47.5 per cent, reacted definitely to orris root. In 6 or 7.3 per cent, it was considered the sole factor. In 33, or 40.2 per cent, it appeared to be only a contributing factor. Any product that is either the sole cause or a contributing factor in 47.5 per cent of perennial hay fever deserves careful consideration. Two case histories relative to this condition will be briefly discussed

CASE 3—E M C, female age thirty-two years has suffered from lacrinating eyes in a moderate degree for three years. She complained of slight itching of the inner canthi of the eye and also had a mild congestion of the mucous membrane of the nose at times with some itching of the nasal mucous membrane. She was advised by a competent eye specialist to be fitted with glasses, although he told her he found no special eye defect. She soon learned that wearing glasses did not change the symptoms of which she complained. Two other oculists were consulted and each fitted her with a pair of glasses but without relief.

Intradermal testing showed a four plus reaction to orris root but a reaction to no other atopic substances

Changing her cosmetics to nonorris root ones relieved her of the lacrinating eyes and the congested nose

CASE 4—L S, female, age thirty five years, came to us several years ago with what appeared to be a chronic infection of the eyes. She complained of a congested and lacrinating nose, which was perennial in type. In July, however, her symptoms were very marked, typical of seasonal hay fever.

Protein tests showed a four plus reaction to both western water hemp and orris root. Changing her cosmetics to nonorris root ones, desensitizing her against orris root with an extract made from it, and treating her seasonal hay fever with an extract made of western water hemp has given her almost 100 per cent relief. Desensitization to orris root has been carried out from ten to fourteen dry intervals over a period of four years. She has tried on several occasions to drop it, but symptoms of what seem to be a nasal cold will always appear. It is interesting to note that a dose of orris root will clear up the symptoms in from two to four hours when they appear.

Case 3 illustrates a type of mild sensitivity to orris root, requiring only the changing of her own cosmetics to nonscented ones and avoiding crowds. Case 4 is a severe one complicated with pollen sensitization.

Careful attention has been given to orris root as a possible factor in the etiology of asthma. Out of 365 asthmatic cases studied, orris root was the sole cause in only one but was considered one of the chief factors in 26, or 7.1 per cent, and a contributing factor in 48, or 10.4 per cent. In other words, it was one of the etiologic factors in 17.5 per cent of all our cases. During the investigation of an asthmatic patient careful testing should be done to rule out, or in, orris root as a possible factor. The following illustrative case histories are presented as evidence.

CASE 5—B B, female, age twenty seven years, came to the clinic complaining of perennial hay fever all her life but worse during the past eight years. Associated with the hay fever symptoms were periodic attacks of asthma. The attacks of asthma had been diagnosed as pulmonary tuberculosis by a number of doctors. It is interesting to note that on putting her to bed in the hospital for rest, her chest findings would usually clear up in from three to six days and her nasal symptoms would be much better.

Both skin and intradermal tests to orris root were four plus in reaction.

Elimination of orris root cosmetics gave but little relief as the routine of her life brought her in contact with others whose bodies and clothing were saturated with scented cosmetics. Desensitizing, however, allows her to attend the theatre and carry on her usual society functions with freedom from either hay fever or asthma.

CASE 6—B M, female, age thirty seven years. Perennial hay fever began as a small child. During the last ten years she has had occasional attacks of asthma.

Protein tests were as follows:

Orris root	+++
Goose feathers	+++
Chicken feathers	++++
Duck feathers	+++

Changing her cosmetics and substituting kapok pillows for the feather ones helped but did not relieve her symptoms entirely. Desensitizing with an orris root extract along with the above procedure gave complete relief.

Both patients mentioned entertain with social functions a great deal, naturally bringing them in contact with heavy concentrations of orris root. Both patients had had a number of nasal operations without relief. In Case 5 orris root was the sole cause of the asthma while in Case 6 it was complicated

with a further sensitization. In most cases of asthma or hay fever due to orris root desensitization is required for relief as removal of orris root from the patient or the patient from orris root is difficult.

Since seasonal hay fever is due to pollen one might feel that orris root sensitivity would play no part in such a disease but to find it a complicating factor is not uncommon. Of 373 cases studied orris root reacted in 27, or 7.2 per cent. In all cases showing a mailed sensitivity it was considered a complicating factor. It is not uncommon to find a patient showing a four plus skin reaction, who has no nasal or bronchial symptoms out of the hay fever season, but as soon as the nasal membranes are made hypersensitive by some pollen then the orris root becomes a definite factor. The following case will serve as an example.

CASE 7—B. M. M., female age forty-two years has had severe hay fever for years but has never received much relief from the use of ragweed extract. She gave a four plus reaction to giant and short ragweed, and nil to orris root. She has no symptoms out of the ragweed season.

She was thoroughly desensitized with ragweed and advised to discontinue the use of scented cosmetics which she did not do. She received about 40 per cent relief from her seasonal hay fever. She finally decided to use no scented cosmetics and found that her hay fever although it was during the height of the ragweed season disappeared.

This is a typical seasonal hay fever case whose orris root sensitivity gave no trouble except after the membranes were made hypersensitive by pollen. There are many such cases. For this reason orris root should always be thought of as a complicating factor in seasonal hay fever.

In the main, my findings are in accord with those of other men working in this field. Cooke¹ found 14.4 per cent of a series of 127 cases of asthma and hay fever sensitive to orris root. Spurr considers orris root an important factor in atopic eczema. Phillips² reported a series of 105 hay fever patients which was made up of both perennial and seasonal cases in which orris root was the only factor found in 7 or 6.6 per cent but it was considered a factor in 26 others or 24.7 per cent. Rackman³ found 10.2 per cent of a series of 428 perennial hay fever cases sensitive to orris root. His findings are in marked contrast to ours. Of the series of 82 perennial hay fever cases which we studied, 40.2 per cent were sensitive to orris root. Rackman however, used the scratch method for testing and the intradermal method was used by us, which in my judgment accounts for the difference.

In a study of the acquisition of specific hypersensitiveness I have shown that a very large per cent of all hay fever and asthma patients are sensitive to more than one substance. It was also noted that the degree of contact with any given protein largely determines whether or not an individual with the inherent ability will become sensitive to that particular protein. The most common protein with which the mucous membrane of the nose and bronchial tubes of the average individual comes in contact is orris root therefore a large number of people, who have inherited the ability to become sensitive, should develop a specific sensitivity to it. As a matter of fact they actually do.

In the year 1926 the women of the United States spent approximately \$200,000,000 for scented cosmetics. Free powders are used very exten-

sively on the face and neck by the average woman and many of them dust themselves all over after bathing either with a scented talc or with some fragrant bath powder. There is a progressive tendency for women to wear less clothing and use more powder, and the scant, loose clothing permits the powder easily to fly in the air. A woman who uses an orris-containing powder soon saturates her clothing to the extent that not only herself but those about her are adequately exposed to orris root.

A reputable manufacturer of cosmetics has informed us that orris root is used rather extensively in the manufacture of a large per cent of face powders, face packs, astringent packs, cleansing powders and creams, scented talcs, bath powders and salts, scented tooth powders and soaps, and that the orris oil is used extensively in preparing most synthetic perfumes and soaps.



Fig. 1—A field of iris plants near San Polo Italy

We have tested some of our orris root sensitive patients and find that their skins are sensitive to most of the scented cosmetics, which is excellent evidence that orris root is present in them.

Orris root is the rhizome of *Iris germanica*, *Iris pallida*, and *Iris florentina*, which is collected in the latter part of the summer, peeled, and dried in the sun. All three species are widely cultivated on the northern shores of the Mediterranean and other parts of Europe. The best quality roots are obtained from *Iris pallida* in Tuscany, and principally constitute the Florentine orris root of commerce. It is, of course, well known that Florentine rhizome is not synonymous with the root of *Iris florentina*, but includes all three varieties.

The district centering around the village of San Polo is renowned for the cultivation of the iris plant. Here practically every farmer grows iris as one of his crops, and from it he obtains the flowers, bulbs, and roots. The bulbs arise from the "eyes" that develop in the root, and vary in number according to whether the plant is a two-year or three-year plant. The flowers are

sold for ornamental purposes, the seeds do not reach maturity, and after cutting the flowers, the root undergoes more rapid development and becomes richer in its aromatic principle.

In San Polo the fields given over to iris have a southern aspect to insure the greatest amount of sunshine. The harvest of the rhizome is in June and August, at which time the season is dry and the roots are well developed. The rhizomes are separated from the bulbs and are immersed in clean water to free them from adhering earthy matter. The bulbs are used for planting, the rhizomes are decocted by the peasant girls and exposed to the sun to dry. The Florentine hills in which San Polo is situated produce by far the greater part of the total production of orris root. The rhizome is worked up for the various orris products in Milan, Grasse and cities in Germany and England where cosmetics are manufactured.

Orris oil is obtained by distilling the various powdered rhizomes with superheated steam and condensing the vapors. A large per cent of the oil of commerce is produced in the neighborhood of Grasse either from rhizomes grown there, or more frequently by distilling the roots imported from Tuscany. These two kinds are easily distinguishable, the French root yielding an oil with a much more delicate violet like aroma. It is used very extensively in most synthetic perfumes and soaps. The function of powdered orris in the preparation of cosmetics is primarily that of a perfumed fixative to make up and hold and slowly diffuse the essences of the scent employed; besides, it has an agreeable odor of its own and a flesh tint.

The methods of testing for orris root sensitivity are the same as those used in testing for sensitivity to pollen, animal epithelium dusts or foods. The skin tests should be used routinely, but in many cases a positive reaction will not be obtained by such a method. For this reason all cases should be checked by the intradermal method which is a much more delicate one. Tests should be well controlled so that errors of interpretation will not be made. The solvent for the orris root should be used in making the control. It is my belief that every asthma and hay fever patient should be carefully tested with orris root, since one sixth of all the asthma and hay fever patients we have examined are sensitive to it.

Hay fever symptoms due to orris root sensitivity vary greatly. Some orris root sensitive hay fever patients give histories which are somewhat similar to those of seasonal hay fever cases but of course there is never any definite relation to the month or time of the year. Most orris root sensitive hay fever patients do not have the marked irritation of the eyes produced by pollen. Their symptoms are usually more marked when on the inside, especially when at church, the theatre or in crowds at any time. Most seasonal hay fever patients can tell the exact time of the year and the exact year their symptoms developed while it is not true, as a rule in the case of the orris root patient, as the onset of symptoms seems to be gradual. Some patients appear to have a great deal more trouble during the summer which is probably due to the increase in powders and cosmetics used by women during the summer months. The warm, dry air of the summer makes the orris root dust more buoyant, thereby allowing a greater concentration of it in the air. The

termination of hay fever symptoms, in those primarily sensitive to orris root, is not at the time of a killing frost as is true of those who suffer from a pollen sensitivity. In some cases the symptoms gradually grow less severe as winter comes on. In other cases, however, the symptoms are much more marked in the winter. The cold air plus the irritation from the orris root, in many cases produces a greater amount of congestion of the nose than does occur in the summer. Symptoms of course, depend to some extent on the occupation, the mode of life, etc. Many women live, during half of the year in the climate of the steam-heated apartment, the average temperature of which is 75° F., and a relative humidity of 19. Such conditions are excellent for the dissemination of orris root if it is used extensively in the home.

It is not uncommon to find orris root sensitive patients also sensitive to pollen, and at certain periods of the summer they suffer severely from typical pollen seasonal hay fever. Most patients who are extremely sensitive to orris root state that they are subject to frequent and sudden colds after being at the theatre church, or any public gathering. In the winter many of these patients will complain of frequent colds associated with itching of the nose, but on careful questioning one will usually find that the general aching which accompanies the average cold will not be present.

There was a time when we advised our orris root sensitive women to refrain from the use of cosmetics, but we soon learned that the vanity of women would not permit such advice to be carried out. It is now our custom to advise those who suffer from an orris root sensitivity to use no face powders, body powders, bath salts sachets or soaps that are scented. A list of the inoffensive powders is given them. In cases that are only moderately sensitive to orris root, such elimination will cure their hay fever symptoms. However, about 75 per cent of the cases are so sensitive that the ordinary routine of life will bring them in contact with a sufficient quantity of orris root to produce symptoms, unless they are specifically desensitized against it. The process of desensitizing is similar to the one used in desensitizing patients who are sensitive to pollen. The treatment in our hands and likewise those of others who are doing special work in asthma and hay fever, has proved very satisfactory. Orris root sensitive patients who complained of frequent colds in the winter who received specific orris root therapy, usually reported freedom from winter colds.

Most doctors who treat hay fever and asthma mention orris root in their writings as a cause. The value placed upon it as a cause of hay fever and asthma by most of them is in accordance with our own ideas. However, until recently few have given it the space it deserves as a causative factor in an ailment which of late years has become distressingly common.

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THE ETIOLOGIC AND SPECIFIC RELATIONSHIP OF FOCI OF INFECTION TO CERTAIN ORGANIC LESIONS A POSTMORTUM STUDY*†

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IN A previous study¹ it was shown that bacteria recovered after death had a definite intravital significance. It became of interest then to determine whether organisms recovered from foci of infection and specific lesions at necropsy possessed a selective localizing power similar to that exhibited by certain bacteria recovered from similar locations during life.

The recognition of the part played by bacterial infections in the causation of chronic diseases has gained in importance with the multiplication of observations on the occurrence of such infections in human beings of ulcers of the gastrointestinal tract and analogous lesions of other organs associated with chronic foci of infection. Certain data for a proper appreciation of the relation between foci of infection and chronic infectious diseases can be more accurately obtained by a systematic bacteriologic study at necropsy than by clinical investigation during life. This is obvious when we consider that only at necropsy are certain regions accessible such as the recessory nasal sinuses, the internal ear, and the central nervous system. These foci are available for direct examination and for cultural studies and it is possible to rule out at once other regions as foci of infection and still further lesions whose etiology is sought can be accurately studied and the pathologic changes other than those in question may be established or eliminated.

Many have contributed to our knowledge of focal infection and the doctrine is now so firmly rooted as to become a tenet. Conspicuous among the contributions is the work of Rosenow² who has gone a step farther by enlarging our knowledge of the selective localizing power of certain bacteria. Focal infection as it occurs in man has been recently reproduced in animals by Rosenow and Meissel. These investigators have established foci of infection in dogs by devitalizing several teeth and filling the root canals with bacteria recovered from patients suffering from nephritis and nephrolithiasis respectively, after a period of from two to four months, similar lesions were found in the dogs.

What determines the specificity of organisms is not clear. However we do know that certain organisms such as the bacilli of tuberculosis and of typhoid, when introduced into a suitable host produce specific reactions in certain organs. We also know that certain organisms have variable invasive

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powers for tissues. As Evans⁷ points out, *Treponema pallidum* may invade the unimpaired mucous membrane of the lip, and the gonococcus the conjunctiva, while both of these structures are quite resistant to invasion by other organisms.

The technic used in culturing solid organs at necropsy has already been described in detail. I shall here describe the procedure used in obtaining the material from foci of infection and from lesions experimentally produced.

When infected teeth were removed, the gums and teeth were washed with 80 per cent alcohol, allowed to dry, and painted with tincture of iodine. The gums were then reflected and external alveolectomy performed with a mallet and chisel. The tooth was then lifted from its alveolus, and if any granulomas were present, they were curetted out and immediately placed in glucose-brain broth. The apex of each tooth removed was snipped off with pincers, or the entire tooth was wrapped in sterile gauze, crushed by a vise, and the root canal cultured. Sterile instruments were used throughout. If free pus was encountered in the socket of the tooth, it was taken up with a capillary pipette, some of this was cultured in glucose-brain broth and on blood-agar plates. The rest was suspended in normal sterile solution of sodium chloride for inoculation purposes. As a routine direct smears were made of the cultured material to rule out contamination.

When organs having relatively thin walls, such as the gastrointestinal tract, were being studied, the desired tissue, such as that of gastric ulcer, was excised with sterile scissors from the mucosa to the serosa and placed in the sterile salt solution and vigorously washed in several changes. If any portion of the tissue was contaminated, it was placed in a 1:1000 solution of mercuric chloride, and after six hours the excess was either washed off in several changes of sodium chloride solution or the sterile saline solution was allowed to run over the tissue for an hour on sterile wire gauze, various surfaces being exposed to the running solution. The tissue was placed in a sterile mortar and ground up with sterile sand, and cultures were then made of an emulsion of the macerated substance with normal sterile sodium chloride solution.

Animals were inoculated either with glucose-brain-broth cultures grown from twelve to eighteen hours, or with a suspension of the infected material in the sterile saline solution. Subcultures were rarely used for inoculation.

The reason for choosing young cultures that were not subjected to prolonged growth on artificial media is obvious when one considers the fundamental principles that govern the pathogenicity of bacteria, as laid down by Pasteur. He demonstrated that old cultures lose their pathogenicity and also that virulent organisms are attenuated when subjected to growth on artificial media and at minimal temperature. Rosenow has gone a step farther and shown that certain organisms, especially those in the pneumostreptococcus group, are very sensitive to differences in oxygen tension and to changes in the hydrogen-ion concentration. He has overcome these difficulties by the use of tall tubes of glucose-brain broth containing a buffer salt. I have adhered to these general fundamental principles closely in my technic under the supposition that changes in the pathogenicity of the organisms would also affect their specificity. Tissues were removed from the heart, liver, kidney,

and spleen, and from all evident lesions found in the animals. These were fixed in 10 per cent formalin, imbedded in paraffin, and the sections cut were stained by hematoxylin eosin and by the Gram Weigert method. The material used for this study comprises cases of gastric ulcer, biliary lithiasis with cirrhosis, and ulcerative endocarditis.

GASTRIC AND RENAL LESIONS

Three cases of gastric ulcer were studied one of which was complicated by renal lesions. The source of material in these three cases was from the teeth which were condemned by the attending physician and the dentist as foci of infection during the life of the patient.

CASE 1—A man aged sixty eight came to the Clinic complaining of involuntary and frequent urination. Initial urinary symptoms occurred five years before and symptoms of duodenal ulcer had been present intermittently for twenty years. The anatomic diagnosis at necropsy included multiple gastric ulcer, duodenal ulcer, marked dental caries and sepsis and suppurative cystoureteropyelonephritis with focal abscesses in both kidneys.

Around two of the six teeth removed aseptically periapical infection was found and in two others infected granulomas. From the apices of the teeth staphylococci and short chain streptococci were cultured. These organisms were also found in the smears and cultures from the granulomatous material. Culture from the kidney yielded chiefly colon bacilli and staphylococci. Some rabbits were injected with glucose brain broth culture of an infected apex of a tooth, others with a normal sodium chloride suspension of the periapical pus and still others with glucose brain broth culture from the patient's kidneys. The following protocols are typical of the results obtained. The lesions and their distribution in the other animals of the series are summarized in the Tabulation.

Rabbits 52 and 53 weighing 1 kg each were injected intravenously with 5 cc of a glucose brain broth culture from an infected apex of a tooth containing almost a pure culture of green producing streptococci and a few colonies of staphylococci.

Rabbit 52 was found dead at the end of thirty six hours. Rabbit 53 was killed by chloroform forty eight hours after injection. A careful, complete necropsy revealed areas of submucous hemorrhage and a clearly defined area of ulceration on the posterior surface of the greater curvature of the stomach extending through the muscular wall (Fig 1). Viewed from the serous surface this lesion was grayish pink and sharply defined by a circle of punctate areas of hemorrhage (Fig 2). Cultures from the blood, spleen and bile were sterile while those from the kidney yielded only green producing streptococci.

Rabbits 54 and 55 were injected intravenously with the pus suspended in normal sterile sodium chloride solution. This pus was obtained from the cavities after the removal of two teeth. Direct smears stained by Gram's method revealed pus cells, staphylococci and streptococci. Rabbits 58 and 59 were each injected intravenously with 5 cc of a twelve hour glucose brain broth culture made from the pus.

Rabbits 54 and 55 died thirty hours later while Rabbits 58 and 59 were chloroformed forty eight hours after injection. Necropsy revealed gastric lesions in all four animals while in Rabbits 55, 58 and 59 there were in addition many focal abscesses in the cortex and medulla of both kidneys (Fig 3). Cultures from the blood and spleen were sterile while those from the kidney and gastric ulcer yielded chiefly staphylococci with a few colonies of green producing streptococci. The two organisms were plated on blood agar and a pure culture of each was injected into five rabbits. It is interesting to note that in the animals injected with the subculture of staphylococci only renal abscesses developed and in the three animals injected with subcultures of streptococci focal hemorrhagic areas were found in the gastric mucosa. On the other hand only in Rabbit 60, that was injected with a pure culture of streptococci not subjected to subculture obtained from the gastric ulcer of Rabbit 58 was a definite gastric ulcer found.

As previously stated the culture from the patient's kidneys yielded chiefly colon bacilli and staphylococci. The colon bacilli were separated and the culture was injected

TABULATION

RESULTS OF ANIMAL EXPERIMENT

CASE NUMBER	CASE ANIMAL NUMBER	ORGANISMS INFECTED	SOURCE	VEHICLE	ROUTE	ANIMAL PASSAGE	EFFECT ON ANIMAL	HEART	LUNGS	STOMACH	KIDNEY	JOINTS	MISCELLANEOUS
1	R52	Streptococci	Tooth apex	G B B *	I†	First	D†	-	+	+	-	+	-
1	R53	Streptococci	Tooth apex	G B B	I	First	Cls	-	+	+	-	+	-
1	R54	Streptococci and staphylococci	Tooth apex	Saline	I	First	D	-	+	+	-	+	-
1	R55	Streptococci and staphylococci	Tooth pus	Saline	I	First	D	-	+	+	-	+	-
1	R56	Bacillus coli	Kidney	G B B	I	First	D	-	+	+	-	+	-
1	R57	Bacillus coli	Kidney	G B B	I	First	Cl	-	+	+	-	+	-
1	R58	Staphylococci and streptococci	Tooth pus	G B B	I	First	Cl	-	+	+	-	+	-
1	R59	Staphylococci and streptococci	Tooth pus	G B B	I	Second	Cl	-	+	+	-	+	-
1	R60	Streptococci	Stomach of R55	G B B	I	Second	D	-	+	+	-	+	-
1	R61	Streptococci	Stomach of R54	G B B	I	First	D	-	+	+	-	+	-
1	R62	Staphylococci	Kidney of R58	G B B	I	Second	Cl	-	+	+	-	+	-
1	R63	Staphylococci	Kidney of R58	G B B	I	First	D	-	+	+	-	+	-
1	R64	Staphylococci	Stomach of R54	G B B	I	Second	Cl	-	+	+	-	+	-
1	R65	Staphylococci	Stomach of R54	G B B	I	Second	Cl	-	+	+	-	+	-
1	R66	Streptococci	Stomach of R54	G B B	I	Second	Cl	-	+	+	-	+	-
1	R67	Streptococci	Stomach of R54	G B B	I	First	Cl	-	+	+	-	+	-
2	R72	Streptococci	Tooth	G B B	I	First	D	-	+	+	-	+	-
2	R73	Streptococci	Tooth	G B B	I	Second	Cl	-	+	+	-	+	-
2	R74	Streptococci	Stomach of R72	G B B	I	First	D	-	+	+	-	+	-
2	R75	Streptococci	Stomach of R72	G B B	I	Second	Cl	-	+	+	-	+	-
3	R2255	Streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
3	R2256	Streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R23	Hemolytic streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R24	Hemolytic streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R25	Hemolytic streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R26	Hemolytic streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R27	Hemolytic streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R28	Hemolytic streptococci	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
7	R29	Hemolytic streptococci	Tooth	G B B	I	Second	Cl	-	+	+	-	+	-
8	R31	Streptococcus viridans	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
8	R32	Streptococcus viridans	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
8	R33	Streptococcus viridans	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
8	R34	Streptococcus viridans	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
8	R35	Streptococcus viridans	Tooth	G B B	I	First	Cl	-	+	+	-	+	-
8	R36	Streptococcus viridans	Tooth	G B B	I	First	Cl	-	+	+	-	+	-

* C B B Glucose brain-broth
† I Intravenous injection
‡ D Animal died
§ Cl Killed by chloroform

intravenously into Rabbits 56 and 57. Both animals were found dead twenty four hours later. Necropsy revealed no gross or microscopic lesions but cultures from the blood, spleen and kidney yielded colon bacilli.

In the kidneys of the patient many focal and conglomerate abscesses were found. Many of the abscesses were relatively recent others were in the

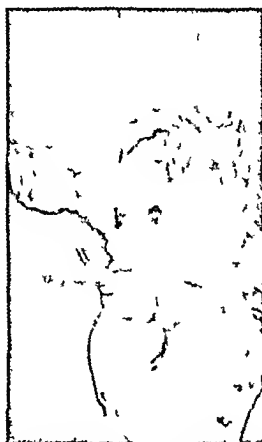


Fig. 1—Rabbit 53 Multiple gastric hemorrhage



Fig. 2—Rabbit 53 Multiple gastric ulcer visible on peritoneal surface

process of repair and still others were apparently entirely organized as indicated by the numerous scarred depressions (Fig. 4). The rabbit's kidneys in which focal abscesses were found correspond to those of the patient in distribution and acute reaction (Fig. 5).

The gastric lesions of the patient were multiple and histologically the base of the ulcers was made up of scar tissue infiltrated with polymorpho-nuclears and lymphocytes. This infiltration extended deep into the muscularis. Sections from the lesions revealed Gram-positive cocci (Fig 6). The ulcers produced in the rabbits were identical in distribution but could not be compared to those found in the patient because they were recent. However, it is interesting to note that two types of lesions were produced. In Rabbits 52 and 53, which were injected with cultures of streptococci, the lesions produced were apparently slight, consisting of areas of hemorrhage, desquamation of the mucosa (Fig 1), and infiltration of the surrounding tissue with polymorphonuclear leucocytes and streptococci (Fig 7). In Rabbits 54, 55,



Fig 3—Rabbit 59 Focal renal abscesses



Fig 4—Necropsy on Patient 2-21 Focal renal abscesses

58, and 59 which were injected with the suspended pus, containing both streptococci and staphylococci, the gastric lesions were different. Grossly the base and surrounding tissues of the ulcer were so markedly thickened as to attract attention to the presence of the lesion before the stomach was even opened (Fig 2), while microscopically the lesion was found to be circumscribed or focal in character (Fig 8) and to contain Gram-positive diplococci (Fig 9).

CASE 2—A man, aged sixty nine, came with symptoms of gastric ulcer. Three weeks previously he had vomited blood. Perforating gastric ulcer was diagnosed. After appropriate preliminary medical treatment, the ulcer was excised by cauterization and posterior gastroenterostomy was performed. At necropsy the chief pathologic lesions were bilateral broncho-

pneumonia purulent bronchitis and chronic diffuse nephritis. Several teeth pronounced diseased at autemortem dental examination were extracted at necropsy and granulomas were removed with strictly aseptic precautions.

Rabbits 72 and 73 were injected intravenously with 5 cc of glucose brain broth culture made from the granulomas. Rabbit 73 was found dead the following morning and was discarded on account of postmortem decomposition. Rabbit 72 was chloroformed forty eight hours after inoculation and necropsy revealed a grayish area 8 by 4 mm on the serosa of the greater curvature of the stomach surrounded by petechial hemorrhages and edema. The corresponding mucosa of this area was found intact. The remaining organs were without evident lesion.

The tissue of the gastric lesion was excised and ground up with sterile sand. This material yielded a pure culture of indifferent streptococci.

Rabbits 74 and 75 were injected intravenously with the culture from the gastric ulcer of Rabbit 72. Both animals were chloroformed three days later and necropsy revealed lesions

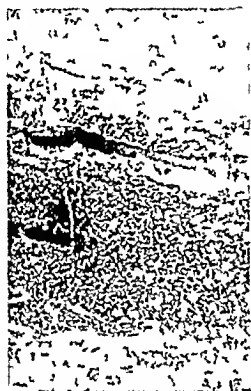


Fig. 5—Rabbit 70. Focal renal abscesses (x10).

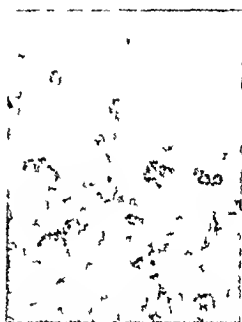


Fig. 6—Necropsy on Patient 221. Gram positive diplococci in base of gastric ulcer (x1000).

similar to those described in Rabbit 2 except that in Rabbit 75 the lesions were multiple (Fig. 2). Microscopically these lesions were characterized by diffuse polymorphonuclear infiltration of the muscular and serous coats while the mucosa was intact.

CASE 3—A man aged forty eight came to the Clinic with a history of symptoms characteristic of gastric ulcer. A large perforating gastric ulcer was excised. The patient died from bronchopneumonia. Culture of the blood, spleen and peritoneum at necropsy yielded a pure growth of hemolytic streptococci.

The roentgen ray and clinical dental reports indicated the extraction of the remaining upper teeth. Accordingly an infected tooth was extracted at necropsy under aseptic conditions and cultures from the apex as well as the pyorrhea pockets yielded pure cultures of green producing streptococci.

Rabbits 2255 and 2256 weighing 2 kg each were injected intravenously with 5 cc of glucose brain broth culture of pus from the alveolus of one of the teeth. Three days later the animals were chloroformed and necropsy revealed punctate hemorrhages with an area of ulceration in the cardiac end of the stomach of both animals. The remaining organs presented no gross lesions.

Comment—In the first passage through animals when the streptococcus alone was injected, only lesions in the stomach were produced. On second passage, when the organism was not subjected to subculture it retained its



Fig 7—Rabbit 53 Gastric lesions showing polymorphonuclear infiltration of submucosa and muscularis ($\times 200$) (see Fig 1)

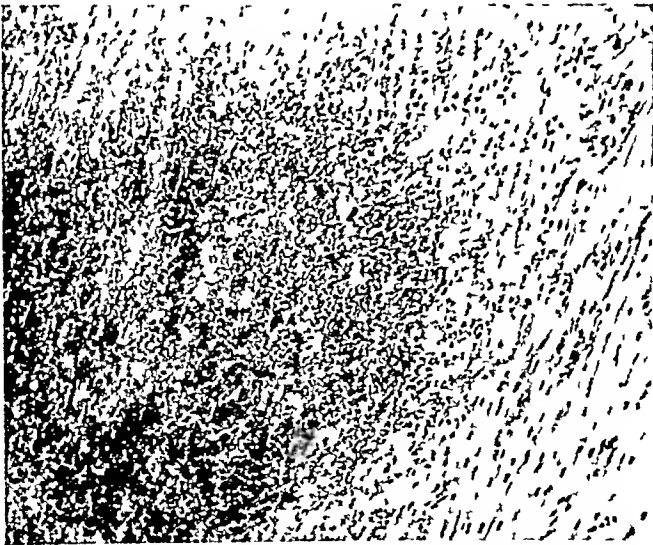


Fig 8—Rabbit 58 Focal necrosis in gastric wall ($\times 100$) (Gross lesion similar to Rabbit 75 Fig 2)

specificity, while, when it was subcultured the lesions were not as frequent and were less marked. When the streptococcus was injected with the staphylococcus, lesions of more marked and extensive character were produced,

while, when the staphylococcus was injected alone it localized almost exclusively in the kidneys and sometimes in the myocardium.

These cases illustrate the value of locating all the lesions which could be of focal origin in a given case. Had only the gastric lesions been known in Case 1 it would have been difficult to interpret the renal abscesses obtained



Fig. 9.—Rabbit 55. Gram positive staphylococci in base of gastric ulcer (x1000).

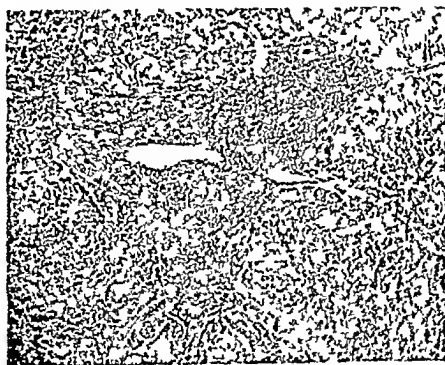


Fig. 10.—Necropsy on Patient 43221. Liver showing marked periportal infiltration with obliteration of some bile ducts (x120).

in the animals. Thus it is possible that in the *intemortem* study of lesions focal in origin, which at times appear irrelevant, may actually have their counterpart in an unsuspected lesion. So far in the study of focal infection the streptococcus group has been given great prominence as an etiologic factor while the staphylococcus has usually been regarded as of relatively little consequence.

That the staphylococcus may enter the circulation from foci of infection in the nasopharynx was suggested by Billings and experimentally demonstrated by Rosenow and Ashby,²⁶ while Israel, Brewer, Jordan, McKenzie and Phemister have reported various lesions, such as osteomyelitis, renal abscesses and myositis, as being secondary to focal lesions in the skin. In reviewing records of 1,000 necropsies I have encountered two cases of diffuse myelitis and three of septicaemia secondary to staphylococcal infection of the skin. Recently Meisser and Rosenow¹⁸ showed experimentally in dogs that when teeth are infected with pathogenic staphylococci from the tonsils in a case of advanced nephritis, extensive lesions of the kidneys were produced. In view of the fact that staphylococci, when injected intravenously, produce lesions of the kidneys and other organs, and that when chronic foci of infection are established with these organisms, they actually enter the circulation, it is obvious that we should give more serious consideration to focal staphylococcal infections.

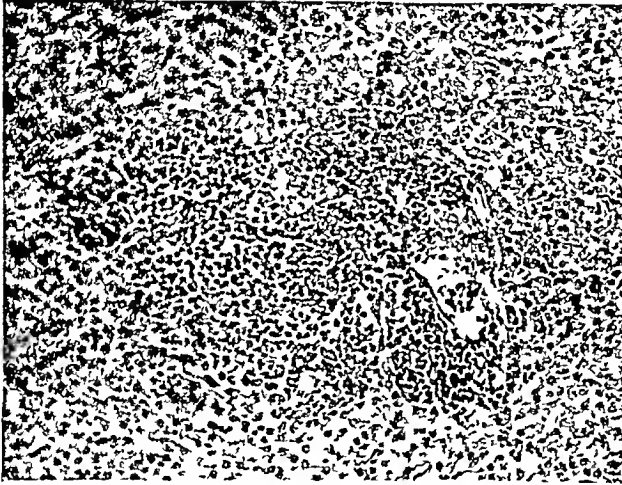


Fig. 11—Dog 2 Periportal leucocytic infiltration involving bile ducts

BILIARY LITHIASIS WITH CIRRHOSIS

Cirrhosis of the liver has, for many years, attracted the attention of the experimental pathologist, but up to the present time, the etiology of this not uncommon disease is as yet obscure. I shall here limit my brief discussion to the relation of bacteria to biliary lithiasis with cirrhosis.

Adam was the first to demonstrate that certain bacteria may cause cirrhosis. In the cirrhotic liver of cattle affected with enzootic disease known as Picton, he found a bacillus which he isolated in pure culture. However, when experimental animals were inoculated with it, they died before any changes occurred in the liver. Weaver, in 1889, isolated a Gram-negative bacillus from an incidental case of infectious cirrhosis of the liver in a guinea pig. This organism, when injected in large doses, killed the animals, but when injected in small doses, it produced a condition closely resembling cirrhosis.

of the liver Heltoen in 1901, isolated a bacillus belonging to the pseudo diphtheria group from a lesion of blastomycetic dermatitis. This bacillus injected subcutaneously produced curdlike changes in the liver. Unfortunately, as is often the case, both of these organisms soon lost their specificity, and complete studies could not be made. Nevertheless in both cases

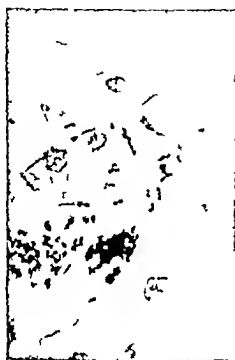


Fig. 12—Dog. Gram positive diplostreptococci in liver



Fig. 13—Rabbit. Gall bladder. Leucocytic infiltration involving entire wall (x100)

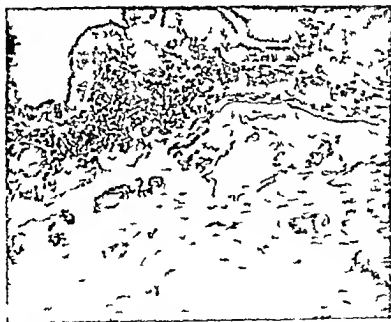


Fig. 14—Dog. Gall bladder. Leucocytic infiltration (x100)

there was sufficient evidence to demonstrate that these organisms had a tendency to localize in the liver and produce a rather characteristic lesion. However, the specificity of some organisms is transient owing perhaps to the unsuitable environment to which they are subjected during their growth on artificial media.

touch the ground with the right limb. The next day it became very ill, and was chloroformed. Scattered throughout the endocardium of the left ventricle were numerous grayish areas varying from 1 to 2 mm in diameter, surrounded by red zones. In the base of the mitral valve and along the line of closure there were raised reddish areas varying from 1 to 3 mm in diameter (Fig 16). The right knee joint was swollen and contained about 3 cc of cloudy fluid. The joints of the right and left fore limbs also contained cloudy fluid. In the smears made from the joint fluid were found pus cells and Gram positive streptococci. Cultures from the blood, spleen, and joint fluid all yielded hemolytic streptococci in pure culture.

CASE 8—This patient had attacks of rheumatic fever with involvement of joints and definite mitral stenosis with auricular fibrillation. Necropsy revealed healed and fresh vegetations along the line of closure of the mitral valve. Two definite infected granulomas were obtained from three teeth. These yielded green producing streptococci in pure culture. Rabbits were accordingly injected. The lesions in the remaining animals injected are noted in the tabulation.

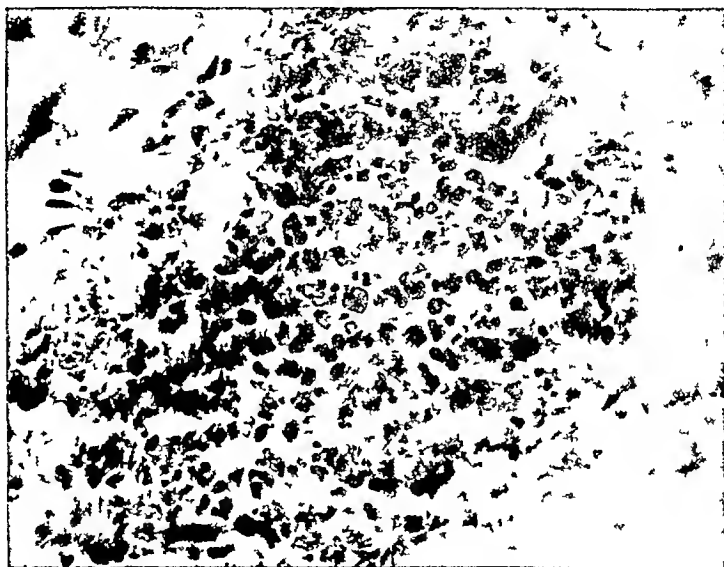


Fig 19—Rabbit R27. Perivascular infiltration of subendocardial nodules ($\times 200$)

Rabbit 34, weighing 12 kg, was injected intravenously with a twelve hour glucose brain broth culture containing green producing streptococci.

Two days later the animal was chloroformed. Necropsy revealed discrete areas of hemorrhage in the endocardium of the mitral valve and left ventricle. In the line of closure of the mitral valve there was a definite area of ulceration 2 mm in diameter, and scattered through the wall of the left ventricle were discrete grayish areas.

Microscopically the punctate grayish areas described in the endocardium of the left ventricle in Cases 7 and 8 were found to extend for a short distance into the myocardium in the intermuscular septa. The areas were focal and made up of groups of large endothelial cells, lymphocytes, and an occasional polymorphonuclear leucocyte arranged close to a vessel (Fig 17). No bacteria could be demonstrated in these areas. The vegetations were made up of necrotic tissue, fibrin, leucocytes and large numbers of streptococci (Fig 18). The lesions found in the myocardium of the rabbits corresponded identically with those of the patient, in being focal in type and

perivascular in distribution, but the predominating cells were polymorpho nuclear leucocytes and lymphocytes (Fig 19) The Gram stain revealed large numbers of diplostreptococci in the center of the lesions (Fig 20)

Comment—The myocardial lesions associated with endocarditis have given rise to considerable discussion in regard to their resemblance to the Aschoff bodies which, according to some authors, are believed to be specific for rheumatic endocarditis Aschoff, in 1904, first described a focal myocardial lesion associated with rheumatic endocarditis and called it "submiliary endocarditis of rheumatic fever" The lesion which he described was multiple and occurred in the intermuscular septa closely associated with a blood vessel it was composed of a group of large oval or spindle shaped cells which occasionally were multinuclear and arranged in the form of a rosette or a fan These cells were surrounded by lymphocytes and a few polymorpho nuclear leucocytes Geipel a year later described similar nodules in cases of rheumatic fever although he did not admit their specificity since he also found them in a case of nephritis associated with interstitial myocarditis Thal

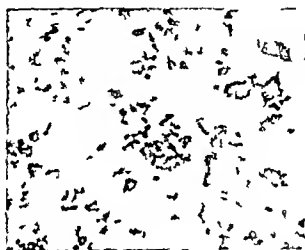


Fig 20—Rabbit R 7 Gram positive diplostreptococci in same area as Fig 19 (x1000)

heimer and Rothschild believe that the presence of Aschoff bodies in the myocardium is strong presumptive evidence of rheumatic fever even though no history is available

The experimental evidence submitted by various investigators is not entirely in harmony Bracht and Wachter maintain that they have reproduced these bodies in rabbits inoculated with streptococci isolated from patients with rheumatic fever The lesions described were focal in character and contained lymphocytes Jackson made a similar study of myocarditis produced by injecting a strain of hemolytic streptococci isolated during a milk epidemic The organism, when injected intravenously had a marked tendency to produce purulent arthritis and focal myocarditis The age of the lesions studied in this series varied from two days to about two months and it is of interest to note that the early lesions contained numerous polymorphonuclear leucocytes and bacteria as well while in the later lesions lymphocytes and oval cells predominated with an occasional giant cell in the old lesions only fibrous scars remained Thalheimer and Rothschild believe that the Aschoff bodies are found only in cases of rheumatic fever and chorea and are absent

in cases of subacute endocarditis, due to *Streptococcus viridans*. They admit that these organisms, when injected intravenously into rabbits, produce focal myocarditis, but they maintain that the cells in these lesions are different in structure and staining reaction with pyronin methyl-green. They believe also that the focal myocardial lesions are of toxic origin since they were unable to demonstrate bacteria. Hartzell and Henrici, in a recent study on the specificity of streptococci, found that a certain percentage of various strains of streptococci of low virulence ingested produce focal myocardial nodules which, although not always of similar character, could not be histologically differentiated from Aschoff bodies, they concluded that the lesion is not specific since it was produced by several strains of streptococci. However, they agree with Thalheimer that the myocardial lesions are of toxic character, since they also were unable to demonstrate bacteria in them.

The two cases cited here represent two different clinical and pathologic pictures: one of vegetative endocarditis (Case 7) and the other, of what is clinically classified as rheumatic fever associated with endocarditis (Case 8). In both cases the manifestations were probably secondary to periapical dental infection, in the myocardium in both cases focal perivascular lesions were found which histologically resemble the submiliary endocarditis of rheumatic fever described by Aschoff. The organisms recovered in each case were culturally different, and when injected intravenously into animals, tended to localize in the myocardium and endocardium, they produced focal, perivascular miliary necrotic areas containing streptococci. The arthritis produced by the hemolytic streptococci was distinctly more pyogenic in character than that produced by the green-producing streptococci.

It would appear, then, from the review of the literature and my own experiments, that in all probability, the Aschoff bodies are not characteristic of rheumatic fever. Perhaps the reason for the disagreement in the comparison of the lesions is that the time factor has not been emphasized. Possibly the earliest lesion is focal perivascular necrosis in which polymorphonuclear leucocytes predominate, due to the chemotactic substances liberated. During this stage, bacteria can be demonstrated. As the lesion becomes chronic, the bacteria are destroyed, the lymphocytes and endothelial cells predominate and eventually only a scar is left.

CONCLUSIONS

1. There is definite evidence that organisms recovered at postmortem examination from foci of infection and specific lesions may exhibit a selective localizing power.

2. Such selectivity is comparable in all its phases, including animal inoculation, with that which has already been proved by other studies. This fact constitutes an additional argument in favor of careful routine and exhaustive bacteriologic examinations of all cases studied at postmortem.

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OCCURRENCE OF LIPOIDS IN URINE AND THEIR DIAGNOSTIC IMPORTANCE*

BY EDWARD L. MILOSLAVICH,† M D, MILWAUKEE, WIS

UNDER the term lipoids we mean fatty substances, which are composed chemically of cholesterol compounds, mainly cholesterol esters, and optically show anisotropic properties. Kaiseiling and Oigler were the first to discover double refracting fat substances morphologically in human tissues, which subsequent investigators recognized as cholesterol esters. At the present time several microchemical methods are at our disposal for their histologic demonstration and differentiation. The above-mentioned authors were the first to observe the presence of lipoids in the urine removed during the autopsy of a nephritic patient.

On this occasion I am considering only that group of fatty substances, which are double refracting, and I am omitting any discussion regarding their chemical nature as well as their microscopic differentiation. For my investigations I employed the Reichert's polariscopic attachments.

The examination of a urinary sediment with this apparatus may reveal the following findings:

1. A minute, double refracting granule, either isolated or found in groups.
2. A cast, consisting mainly of anisotropic material, which is termed lipid cast.
3. Small epithelial cells, apparently desquamated cellular elements of the tubular apparatus of the kidney, which contain double refracting substances in their protoplasm.
4. Larger, foamy cellular elements including anisotropic fat substances, derived from blastomatomatous growths in the genitourinary tract.

The more cholesterol esters are present in a fat globule, the more pronounced will be the anisotropic phenomenon, and the sharper will be the polarizing cross figure.

The first question to be answered is: Do the normal renal parenchyma and the normal urine contain double refracting substances?

The exact and extensive histologic investigations of several research workers proved that lipoids occur in neither the tubular apparatus nor in interstitial tissue of the normal kidneys. Personally I have examined the urine with polariscopic attachments in 380 cases of both male and female individuals in various decades of life, who did not present clinically any renal symptoms and whose urine when chemically analyzed was normal with the exception of an occasional faint trace of albumin, in no case did I succeed in de-

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teeting double refracting substances in the sediment Von Noorden, Munk, and Genel, also emphasize the fact that in normal urine no cholesterol esters can be found

One must admit theoretically however that under normal conditions the blood cholesterol may be excreted through the kidney (alimentary transitory lipodemia), but in such minute quantities as to defy detection completely, consequently this occurrence would not carry with it any practical significance

Even during certain pathologic conditions which are accompanied by a hypercholesterolemia we are not able to observe lipoids in urine as long as the kidney is not affected In connection with this fact the experiments of Weltmann and Biach and Genel are of particular importance since during the course of an artificial hypercholesterolemia these authors succeeded in finding lipoids in urine, only after the kidney had been previously damaged with mercurum nitricum The occurrence and ready detection of lipoids in urine, therefore represents a pathologic condition which is called lipoiduria or cholesteroluria

Now the question arises under what circumstances can lipoids be found in urine?

In the first place and mainly in the so called genuine lipid nephrosis, a peculiar nosologic entity accurately described by Munk characterized by the development of a general edema marked albuminuria hypercholesterolemia and lipoiduria, by the absence of high blood pressure and hematuria The urine sediment is loaded with double refracting fat substances, lipid casts and with lipid bearing cells It is most likely a metabolic disease a primary disturbance of cholesterol metabolism its real nature being still undetermined

Certain cases of lipid nephrosis may develop acutely during the secondary stage of syphilis, as it was originally pointed out by Munk and Fahr The urine sediment at this time is rich in double refracting substances and lipid casts These findings in combination with a nephrotic symptom complex and a positive Wassermann blood reaction characterize an acutely developed primary lipid nephrosis In the blood we may also find an increase of lipoids probably indicating a disturbance in lipid metabolism

Occasionally, however during the salvarsan mercury treatment toxic lesions of the kidney may suddenly occur, with a subsequent appearance of lipoids in the urine These instances must be sharply separated from the cases of primary lipid nephrosis The differential diagnosis must be based upon an exact clinical analysis and observation (functional kidney test)

Lipoids in urine may also occur as it was first emphasized by Munk in chronic and subacute cases of glomerulonephritis in fact approximately eight weeks after the acute onset, but never during the acute stage This indicates that nephrotic changes start to accompany the nephritic process In certain cases of genuine and atherosclerotic shrinkage of the kidneys lipoids may appear in urine, their occurrence being uncommon even irregular Munk states further that in cases of acutely developing parenchymatous degenerative changes of the kidneys urine does not contain double refracting substances

From these observations and contentions we may advance the following rule The presence of lipoids in urine in a case of a pure nephrotic symptom complex proves a primary genuine lipid nephrosis In nephritic cases, however, their appearance in urine signify secondary degenerative or infiltrative deposits of lipoids in the tissues of the kidney, a secondary lipid nephrosis

Dr F D Murphy, who, upon my suggestion, has made considerable clinical and pathologic studies of cholesterol in various forms of nephritis, points out, that those cases of chronic diffuse glomerulonephritis which were associated with edema, were characterized by the presence of lipoids in the tubular epithelium of the diseased kidneys But on the contrary, those cases which were not accompanied by edema did not disclose any deposits of anisotropic substances in the cellular elements of the renal tubules Murphy believes that an association exists between glomerulonephritis with edema and an elevation of the blood cholesterol, deposits of lipid material in the tubular epithelium, and the presence of double refracting substances in the urine His investigations are in complete accord with the findings of Munk In a series of fifty cases of heart disease with edema Murphy was unable to find any abnormal amounts of cholesterol in the blood and no lipoids in the urine, indicating herewith the possibility of differentiation between a cardiac and a renal diopsy

Several authors (Finger and Kollet, Genck, Knack) questioned the statements of Munk regarding the absence of lipoids in urine in cases of acute glomerulonephritis since they apparently succeeded in finding double refracting substances in the urinary sediment during a very early stage It is, however, questionable whether these investigators were absolutely familiar with the exact time of the commencement of the acute nephritic process

A further pathologic condition of the kidney, in which the lipoids may play an important diagnostic rôle, is amyloidosis In amyloid degeneration of the kidney, in amyloid nephrosis, we meet with cases which show a rich deposit of double refracting substances in the interstitial tissue of the kidney and very negligible amounts in the renal tubular apparatus On the other hand we know of instances, where mainly the cellular elements of the tubules contain lipoids, occasionally to such an extent that one can easily recognize and follow the structure of the kidney parenchyma in the dark-field during the micropolariscopic examination The latter cases are clinically accompanied by a nephrotic symptom complex and by a cholesterolemia

In connection with this statement I want to mention the observation of Fahr, who found large deposits of cholesterol esters in the kidneys in each of his nineteen cases of amyloid nephrosis with edema, while the double refracting substances were absent in cases without edema

The amyloid nephrosis can be assumed with the greatest probability if, during the course of certain wasting diseases (such as chronic purulent affections of bones or joints, severe ulcerous types of tuberculosis, persistent bronchiectatic suppurations, etc), the nephrotic symptom complex appears We can, therefore, clinically differentiate between a simple amyloid degeneration of the kidneys and an amyloid nephrosis

Certain types of malignant neoplasms of the urogenital tract may occasionally lead to lipoiduria, as the so called hypernephroma or Grawitz tumor

of the kidney, especially in those instances, when the tumor mass perforates into the pelvis of this organ. The tumor tissue is rich in fatty substances, which partly exhibit anisotropic character. Their detection in urine, in combination with clinical manifestations may be of some help to the urologist while endeavoring a diagnosis.

A peculiar kind of primary carcinoma of the prostate, known as lipid carcinoma, may produce lipoiduria. This tumor variety was first described by Schlagenhauser, a Viennese pathologist. I had the opportunity to examine a similar case three years ago at St. Joseph's Hospital Milwaukee, Wis. If the tumor penetrates into the lumen of the bladder, the urinary sediment may contain tumor cells which are loaded with lipoids and also isolated double refracting substances in the form of minute granules.

In adult male individuals, particularly in advanced age the prostate contains lipoids under normal conditions which are found in the epithelial elements as well as in the interstitial tissue. The same is true in many instances of the so called hypertrophic adenomatous prostate. Dr. W. M. Kerns examined under my direction a large series of such prostates and confirmed these findings. In my own micropolariscopic examinations I did not succeed in finding lipoids in urine in cases of so called prostatic hypertrophy.

Kinger and Kollat have apparently seen lipoids in the urinary sediment in a case of pyelonephritis and are inclined to question their clinical diagnostic importance. Far more significant is the fact that Genck investigated thirty three cases of cystitis pyelitis and cystopyelitis and found only negative results.

Lately during postmortem examinations of two cases which showed a pronounced hypercholesterolemia, I found yellow, xanthoma like nodules, about the size of a pea in the choroid plexus of both lateral ventricles consisting mainly of cholesterol crystal formations which I designate as cholesteroma. Sorry to say I neglected to examine the lipid content of the cerebrospinal fluid. These findings may lead us to believe that these tumor like cholesterol deposits in the choroid plexi are probably concomitant with increased presence of lipoids in this fluid influenced by a disturbance in cholesterol metabolism.

While summarizing these brief statements permit me to emphasize that lipoiduria, the occurrence of double refracting fat substances in urine occurs in the following pathologic conditions:

- 1 Lipoid nephrosis of unknown origin (genuine lipoid nephrosis)
- 2 Lipoid nephrosis of luetic etiology (secondary stage of syphilis)
- 3 Subacute and chronic glomerulonephritis (combination forms of nephritis and nephrosis)
- 4 Amyloid nephrosis with edema
- 5 Grawitz tumor (so called hypernephroma) of the kidney
- 6 Lipoid carcinoma of the prostate

I have presented only a brief outline of that which one could consider on this very important subject. In reviewing the textbooks of clinical laboratory methods we are unable to find any information about this matter. My

aim was to arouse the interest of clinical pathologists in this question and to stimulate further observations and studies. I want further to emphasize the practical importance of the examination of the urinary sediments with polarizing microscope for clinical diagnostic purposes, and to recommend it as a routine procedure for every pathologic urine.

DISCUSSION

Dr Wm G. Exton—This matter is a very important one. We have extracted a number of albuminous urines with alcohol and ether, and I have been surprised at the number of urines from which fat could be extracted by simply shaking them with ether or alcohol and ether.

Dr Mortimer Heitzberg—I wish to know if Dr Miloslavich has any observations on the evidence of fat in a tuberculous kidney or in conditions of pernicious anemia.

Dr Wm G. Exton—It has been confusing to me that lipoids might be found in the urine which do not show in the sediment. In a number of instances we have not been able to see them in the sediment but have been able to extract them with alcohol and ether. The presence of lipoids in urine is certainly worthy of investigation, and I hope Dr Miloslavich will pursue his studies and further enlighten us as to methods and significance.

Dr Miloslavich (closing)—The technique is a very simple one, the polariscope attachments can be applied to any microscope. The urinary sediment is then used in the ordinary way. One point of interest would be to investigate whether or not the temperature of the urine influences the demonstration of the presence of lipoids. In cases of pernicious anemia and advanced tuberculosis, one may find presence of isotropic fat substances.

INCIDENCE OF VARIOUS SPECIES OF BACTERIA IN SPINAL FLUIDS FROM CASES OF MENINGITIS*†

By RUTH GILBERT, M.D. AND MARION B. COLEMAN, B.S., ALBANY, N. Y.

A COMPILATION of the results of the routine examination of spinal fluids over a period of seven years has been made in order to determine the incidence of various species of bacteria in spinal fluid from cases of meningitis in New York State outside of New York City. As the district served is largely rural, it is of interest to compare our figures with those furnished by Neal¹ and Dunn² who have reported specimens from cases in urban districts.

Since January 1, 1920 in two hundred and eighty four instances organisms of pathogenic significance have been isolated. The figures in Table I represent the number of patients from whom specimens were submitted two or more specimens frequently having been received from the same person.

TABLE I
SPECIES OF BACTERIA ISOLATED FROM CASES OF MENINGITIS

REPORTED BY	TUBERCLE BACILLI	MENINGO COCCI	B. INFLU ENZAE	PNEUMO COCCI	STREPTO COCCI	OTHER BACTERIA	TOTAL CASES
Neal, New York City	662	62	51	86	83	16	1523
Dunn, Boston	60	60	4	12	6		142
Gilbert and Coleman, Albany	167	18	23	23	35	8	284

The number of tuberculous spinal fluids (167) is relatively very high. The age incidence, however, corresponds to that determined by others, eighty nine of the patients being under ten years of age. A study of the specimens from this group of children under ten years of age has been made to determine whether the disease was of bovine or human origin. Rabbit inoculations with 0.01 mg. amounts of culture in thirty one instances have indicated ten strains to be of bovine origin and twenty one of human origin. The study of the other strains has not been completed.

The relative percentage of specimens from which meningococci were isolated is very low. Of the eighteen strains reported, there were three corresponding serologically to Gordon Type I, three to Type II, one which was agglutinated equally well in Types II and III sera, and one which was agglutinated in all three sera. Seven others were morphologically and cultur-

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ally typical of meningococci and were agglutinated definitely in the polyvalent meningococcus serum, but were not agglutinated in Gordon Types I, -II, or -III sera. These would be classed as "X" atypical, corresponding to the strain reported by Knikbide and Hutton³ in 1926. The three remaining strains were classified as meningococci because they had morphologic and cultural characteristics of meningococci and appeared to be the incitants of meningitis. They were not agglutinated in polyvalent meningococcus serum. The fact that meningococci isolated from sporadic cases of meningitis are serologically less uniform than those found in an epidemic, has been frequently reported by other workers. The relative infrequency of the isolation of meningococci may have been due to the length of time, usually one or two days, which our specimens are in transit. However, specimens containing large numbers of polymorphonuclear leucocytes in which no bacteria can be demonstrated are rarely received, and in only four instances were morphologically typical meningococci found in the spinal-fluid sediment when they were not found on cultural examination.

Pfeiffer's bacillus was found in twenty-three instances and, as noted by others, the majority of cases (17) occurred in children two years of age or less. There was no indication in the histories accompanying these specimens that the meningitis followed any type of respiratory disorder except that two were from children who were recovering from whooping cough. As is generally known, the mortality of influenzal meningitis is high. In a review of two hundred and twenty cases, Rivers⁴ reports only seventeen recoveries. It has been our experience that this type of infection is definitely indicated by the presence of large numbers of very pleomorphic Gram-negative bacilli, together with large numbers of polymorphonuclear leucocytes in the spinal fluid sediment, and a tentative diagnosis may be made on these findings. The work of Wollstein⁵ and Rivers and Kohn⁶ indicates that the strains of B. influenzae found in meningitis are closely related serologically. Serum treatment in experimental influenzal meningitis in monkeys was used by Wollstein⁷ with satisfactory results. Torrey⁸ reports the recovery of two cases in children after serum treatment, Neal,⁹ one after vaccine and serum treatment, and Jousset and Guard,¹⁰ one after vaccine treatment alone. Therefore, it would seem that if satisfactory serum or vaccine treatment could be perfected, the ability to make an early diagnosis would permit its effective use.

Pneumococci and streptococci were found in thirty-three and thirty-five instances respectively. In only three of the pneumococcus infections was a history of pneumonia given. Sixteen of the pneumococcus strains isolated were Type IV, thirteen, Type III, two, atypical Type II, and two, Type I. Among the specimens containing hemolytic streptococci were two from meningitis following head injuries, and three, following mastoid infections. Seven of the streptococcus strains were of the variety producing methemoglobin. These are included because this organism was present in pure culture and was found in the original film preparations of the sediment.

In a few specimens organisms not commonly associated with meningitis were found, i.e., B. typhosus in one instance (the history did not indicate

whether this was a typical case of meningitis or one of typhoid showing meningeal irritation) *Micrococcus enteritidis* in two instances and *B. proteus* from a patient with meningitis following a fractured skull. In four other instances, unidentified organisms were found, their presence possibly being due to contamination.

In conclusion it is important to note the relatively low incidence of meningococci in this series which is lower in fact than that of *B. influenzae*. As the mortality in cases of meningitis due to *B. influenzae* is very high and there is some evidence that vaccine or serum treatment might be of value a further study of such agents may be indicated.

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LABORATORY METHODS

A NEW AND RAPID METHOD OF EXAMINING TISSUE MICROSCOPICALLY FOR MALIGNANCY*

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INTRODUCTION

NEW Method—Not all tumors can be diagnosed in the gross. Even our best surgeons need from time to time a quick microscopic examination for malignancy. Up to the present the most rapid method has been that of frozen sections. In the hands of those who have acquired the necessary skill and experience, frozen sections are dependable. But against their use many objections have been raised. It is feared that the freezing may produce misleading artefacts. The method is expensive, and the services of an expert technician are required. Even under good conditions the sections are sometimes torn, wrinkled, or distorted. As a consequence of these and other objections, many experienced pathologists do not use frozen sections at all. There is a real need for some easier method which will permit a quick microscopic diagnosis of tissue and yet will avoid most of the objections raised against frozen sections. In this paper such a method is described.

HOW METHOD WAS EVOLVED

The new method is the outcome of experimentation stretching over a period of nearly eight years and is the direct result of a visit paid the Mayo Clinic in the summer of 1919. At that time Dr. William Carpenter MacCarty showed me the most beautiful frozen sections of fresh unfixed tissue I had ever seen. The technique employed had been introduced in 1905 by Dr. L. B. Wilson,¹ and the stain was Unna's polychrome methylene blue. Before the war, according to Dr. Wilson, the best samples had been obtained from Germany, but at the time of my visit it was almost impossible in this country to purchase good German stain. To ripen it at room temperature I was told required from six months to a year or more, and many batches spoiled in the ripening.

As I wished to use the stain, I tried to learn how to ripen it more rapidly. Thus I succeeded in doing by incubating the alkalinized stain at 37.5° C. for six days, and this method was adopted at the Mayo Clinic.² Further experiments showed that at a higher temperature the time could be reduced to a few minutes. The stain can now be ripened by boiling for sixty seconds.³

Alkaline polychrome methylene blue is excellent for frozen sections of fresh unfixed tissue, but is not so satisfactory for frozen sections of fixed tis-

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sue As most of the tissues I examined were fixed in formalin, experiments were carried out to produce a stain that would be equally satisfactory on fixed tissue These experiments were also successful⁴ Subsequently it was found that by altering the concentration of the stain for fixed tissue, it too could be ripened by boiling for sixty seconds

The satisfactory results obtained with these two stains on frozen sections of fresh and fixed tissue suggested their use on tissues studied in the gross As a consequence thick slabs of tissue were cut, stained, and examined by reflected light The results were surprisingly good With low powers of the microscope small areas of malignancy could often be picked out In many instances, however, the low powers merely raised a suspicion of malignancy To be certain of the diagnosis higher powers were essential

Improvements in technique permitted magnification up to 100 or 150 To make this possible, special lamps⁵ were constructed and so attached to the microscope that a beam of strong light was directed obliquely downward upon the upper surface of the stained sections For assistance given me at this stage of my work I wish to acknowledge with thanks my great indebtedness to the optical firms of L. Bertz in Wetzlar, Germany, and Bausch and Lomb in Rochester, New York These firms designed or adapted lamps that I could easily and successfully use

For a time however all attempts to get satisfactory magnification above 150 diameters were in vain An accidental observation eventually led to success and to the discovery of what I am inclined to believe is a new principle in microscopy One day I was struck by the fact that occasionally some thick stained sections examined by transmitted light were ever so much better than others On attempting to find the explanation all of the good sections were carefully examined, and it was found that these had one thing in common All showed one side well stained but the other side was stained poorly or not at all This irregularity in staining was accidental but to determine its significance I now stained other sections taking care to have the stain on one side only The result was most gratifying Thiel sections stained on one side only and examined by transmitted light often showed nuclei and other histologic details as satisfactorily as if they were very thin sections The explanation seemed simple If the sections are stained on one side only and very superficially light can easily be transmitted through the other unstained side thus causing the stained cells to stand out sharply on an illuminated background On the other hand if the section is stained on both sides, light transmitted through the first surface is altered in quality and it may be so completely absorbed that too little gets through to illuminate the upper surface sufficiently The correctness of this explanation has apparently been amply demonstrated by the tests to which it has been subjected

New Principle—Instead of having to cut tissue very thin to get histologic detail by this new principle all that is necessary is to stain relatively thin sections superficially on one side only with a good precise stain and then examine the moist tissue, stained side uppermost under a thin cover using transmitted light With fixed tissue astonishingly beautiful microscopic pictures were obtained⁷

Unfixed Tissue a Problem—When, however, the same technique was applied to fresh tissue, the results were not satisfactory. Unfixed tissue is usually so soft that it is difficult to section. Moreover, under the microscope the stained surfaces often showed numerous unstained areas. The rapid microscopic diagnosis of unfixed tissue presented a real problem.

Two possible solutions suggested themselves. (1) Some new method of rapid yet perfect fixation might be worked out, or (2) the technique for fixed tissue could in some way be altered to make it suitable for fresh unfixed tissue.

Quick Fixation—The simpler and easier solution seemed to lie in quick fixation. It is well known that heating fresh tissue for a short time in formalin often enables one to secure satisfactory results if frozen sections are cut and stained with hematoxylin and eosin. But polychrome methylene blue is a very sensitive stain. For it to act well, the tissue apparently must either be unfixed or well fixed. Its use on specimens heated in formalin seemed to indicate that tissue that was *quickly* fixed was *imperfectly* fixed. With none of my many attempts at rapid fixation was I well satisfied.

Characteristics of Unfixed Tissue—With a view to adapting the fixed tissue technique to fresh tissue, the characteristics of the latter were studied. Fresh unfixed tissue differs from fixed tissue in at least five ways. (1) It is usually softer. To cut it properly the razor should be as sharp as possible. (2) The cells of unfixed tissue are much easier displaced or dislodged. If after staining some are displaced, the surface under the microscope will show areas which appear to be unstained. To avoid losing cells from the surface, the greatest care must be exercised in cutting, staining, washing, and mounting. (3) Unfixed tissue frequently decolorizes the stain. If the stained tissue is to be seen at its best, it must be examined at once. (4) Unfixed tissue is more translucent than fixed tissue. This is a great advantage, for it permits transillumination of thicker sections. (5) Some fresh tissues are very red due to the presence in them of many red blood cells. Red tissues are difficult to examine satisfactorily, for the blue rays in the examining light are absorbed.

New Technique—With the characteristics of unfixed tissue in mind the technique has been modified until it is now applicable to many tissues, although not to all. The details of this new technique will be given after the advantages and disadvantages of the new method have been pointed out.

ADVANTAGES

Quickly Learned—The technique is simple, easy, and quickly learned. Anyone with good eyesight, intelligence, and a little mechanical dexterity can acquire the necessary technique in a few minutes.

Extremely Rapid—In favorable and easy cases the microscopic diagnosis can frequently be given in less than sixty seconds.

Uses No CO₂—As no CO₂ is required, the usefulness of the method is widened. It can be employed where no CO₂ can be secured, where the supply is exhausted, where the services of an expert frozen-section technician are not available, or where the freezing apparatus is out of order.

Inexpensive—The new method is inexpensive. If one already has a microscope the other things necessary can be secured for a few dollars, and the cost of operation and upkeep is almost nothing.

Transportable—The equipment is simple, compact, light, and readily transportable. The method seems ideal for use in the doctor's office, or in an emergency in the patient's home.

Noiseless—As the method is noiseless it can be used in the operating room. For this purpose it seems to be the best yet devised.

Timesaver—The new method saves the time of the surgeon, the pathologist, the technician, and the patient.

1. The sooner in doubtful cases the surgeon receives his tissue report the sooner he can complete his operation. If the surgeon is promptly assured that a given piece of tissue is or is not malignant his operation is planned and carried out on the basis of definite information. If he can feel sure that malignancy is not present he can often substitute a simple and less radical operation for the one he would have done if he had not received a very prompt tissue report. The new method is apparently the fastest yet proposed.

2. As a rule the pathologist cuts enough blocks of tissue to be reasonably sure that in one or more he will find the changes necessary for his diagnosis. The more difficult the case seems the larger the number of blocks taken. Often eight or ten are excised when one would suffice if the pathologist could know at once what it shows. By the new method a microscopic diagnosis can be secured on each block as it is taken. The pathologist then preserves only those which are of value. As his diagnosis is reached sooner he saves time and tissue.

3. By having to section or embed for permanent record a smaller number of blocks much of the technician's time is saved. There is also a big saving in reagents, slides, and covers.

4. Patients wish to get well quickly and be out of the hospital as soon as possible. If in a number of cases the new method permits the surgeon to perform less radical operations the time and other interests of the patient are conserved.

Few Artefacts—The method is comparatively free from artefacts. The tissue is examined perfectly fresh. There is no freezing, fixing, heating, or dehydrating. Probably many of the cells are still alive. In any event smooth muscle in the stained sections often shows lively contraction. By this new method cells are studied as nearly as possible as they are in the living body. This is a great advantage.

No Special Lamp—With the new technique no special lamp is necessary. Under favorable conditions strong daylight suffices, but a good, efficient artificial light is better.

High as well as low powers of the microscope may be employed. If the technique is good, oil immersion examination of the tissue is possible and satisfactory.

Dependable—The method gives dependable microscopic pictures. When the technic is carefully carried out and the diagnoses are made by a skilled and experienced pathologist who, until he is thoroughly familiar with the new method, controls all of his diagnoses by the older methods, there is little occasion to fear that malignancy will be missed, or that it will be diagnosed where it is not present.

Main Value—While it is probable that the new method will prove to be most useful in studying malignancy, it is by no means limited to this. Acute and chronic inflammatory changes are often clearly seen. The method should be of great value in selecting tissues to be examined by other methods, in studying tissues from autopsies, in checking doubtful diagnoses, and in identifying unlabelled tissues.

Advantages of New Stain—By substituting for the alkaline stain a neutralized polychrome methylene blue, the method here described works very satisfactorily both on fresh and on formalin fixed tissues.⁷ This is a great advantage, for fixed tissues are often easier to cut and diagnose than some fresh unfixed tissues.

In Studying and Teaching—The new razor section method should prove of value in the study and teaching of cytology, histology, and pathology. It should also be useful on fixed tissue. Students of pathology often wish to see sections of normal tissues. By keeping on hand portions of normal organs fixed in formaline, it is easy to cut, stain, and mount sections of them whenever they are called for.

Tissue Not Wasted—The preliminary cutting of razor sections does not prevent the use subsequently of other methods of diagnosing. Even thin razor sections are usually thick enough to be cut on the freezing microtome or embedded and cut in paraffin or celloidin. Moreover, the staining of sections with polychrome methylene blue does not interfere with the subsequent staining of these with other stains, for the polychrome is extracted completely when the tissues are run through alcohol.

DISADVANTAGES

The new razor section method for fresh unfixed tissue has a number of disadvantages.

Not Permanent—As the tissue is not fixed, the sections are not permanent. In this respect they resemble frozen sections of fresh unfixed tissue. To secure permanent sections some other method must be employed.

Quick Fading—The sections fade very quickly. They must, therefore, be examined immediately after staining. The sooner they are seen, the more beautiful they appear. Faded sections can, however, be repeatedly restained.

Limited Applicability—While the method is suitable for the examination of the majority of unfixed tumors, it is not now easily applicable to all. At present it is also unsatisfactory for examining some unfixed normal tissues. This limitation in applicability is the method's greatest disadvantage. With further improvements in technic, it is to be hoped that this drawback will be reduced or overcome entirely.

Difficult Tissues—When examined in the fresh unfixed state, tumors and other tissues may be difficult for five reasons. They may be hard to cut, stain, mount, examine, or diagnose.

1 They are hard to cut if they contain much calcium or bone, if they are extremely soft, or small, or friable, or if they are composed of numerous small polypoid masses.

2 They are hard to stain if necrotic or if they are covered with mucus or colloid.

3 They are hard to mount if very small or extremely narrow or ragged or if the tissue is composed largely of living smooth muscle. This latter by its contraction, often throws smooth sections into many folds.

4 They are difficult to examine if light cannot be readily transmitted through them. Opaque tissues or those which are darkly pigmented are difficult to transilluminate. Moreover tissues that are red are also difficult. Very porous tissues and sections which have rough surfaces due to improper sectioning act in a similar way for they allow the stain to sink quickly into the depths. When in these cases transmitted light is used, it has to pass through a stained layer before it reaches the upper surface and this stain in the depths acts like a color screen, absorbing most of the light.

5 They may be hard to diagnose due to inherent difficulties. Border line tumors often necessitate prolonged study. As razor sections stained by polychrome methylene blue fade quickly it does not seem probable that this new method will be especially useful here although in its favor is the fact that it permits the rapid study of many sections from different parts of the tumor.

Not Projectable—Razor sections cannot yet be satisfactorily projected. They are too thick and they fade too quickly. On account of the rapidity with which they are decolorized by strong light it is even difficult to get good microphotographs.

Reasons for Fading—For the rapid fading of the stained razor sections there are apparently three reasons. (1) As the sections are relatively thick and the staining is at first very superficial the unstained cells in the neighborhood of the stained ones soon begin to rob them of stain. (2) many living tissues quickly decolorize the stain. (3) the light used to examine the sections also decolorizes them.

Restaining—Sections that have been decolorized more or less completely can be restained. The restaining can be repeated several times, but eventually becomes unsatisfactory due to the tissues in the depths becoming stained.

This list of disadvantages is long, but it should not discourage for most tumors and many other tissues are easily examined. If there is no hurry in making the diagnosis, the difficult tissues should be fixed in formalin and examined again by the razor method.

Interesting Parallelism—It is worthy of note that the cases which have been difficult by the new method have, as a rule been difficult also for frozen sections. Sometimes one method shows a little advantage sometimes the other.

TECHNIC

The technic described in this paper is the result of many experiments. If the directions here given are followed carefully, satisfactory sections should be obtained.

Equipment—This is simple and inexpensive. In addition to the microscope the following things should be on hand. A good sharp razor, a hone, and a strip. A small piece of cork measuring about 10 by 10 by 0.5 cm. or larger, some pins, and a good pair of pointed forceps capable of picking up a hair. One or two tumblers full of distilled water or of clear cold tap water, a bottle of good neutral polychrome methylene blue, a medicine dropper, a good strong artificial light, some clean cover glasses and microscopic slides, and one or more slides of the same size, made of glass or wood, but

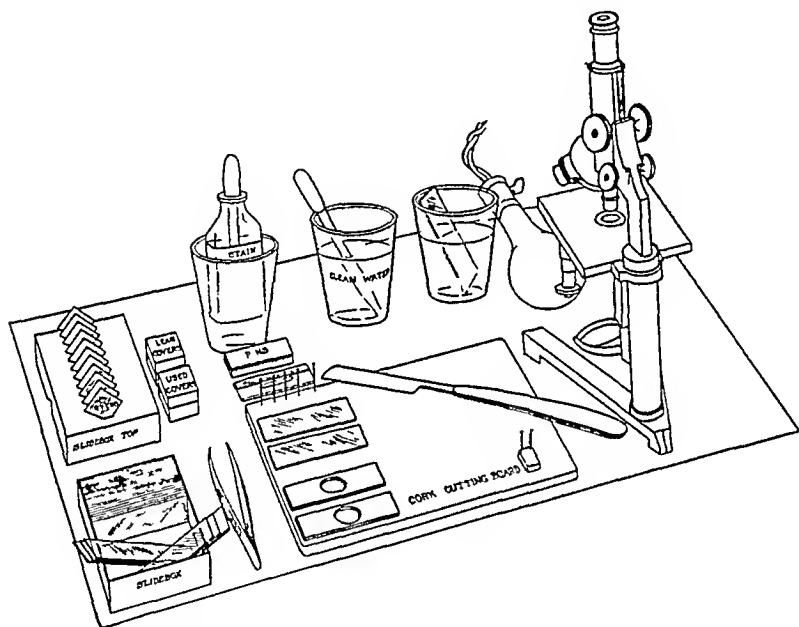


Fig. 1—A simple but efficient arrangement of the inexpensive equipment

differing from the ordinary slides in having a round or oval hole 2 cm. or a little larger cut entirely through the center. These special slides are convenient but not necessary.

The razor should be kept as sharp as possible. Whenever it becomes the least bit dull, it must be honed and stropped. An experienced barber can quickly teach you how to sharpen it effectively. The importance of keeping the razor very *sharp* can scarcely be overestimated. The kind of razor is less important. I have used a number of different ones and all have been satisfactory if *sharp enough*. Even safety razor blades and microtome knives have been tried. The Duham Duplex razor blade is convenient and is safe to use if one edge is first covered over with a little adhesive plaster to lessen the danger of the operator cutting himself. The Sextoblade is also excellent. Best of all, however, in my experience is the old-fashioned razor which is still used by most barbers.

A thin piece of cork is used as a cutting board. Into this cork, pins are stuck to immobilize the tissue. A table mat of compressed cork is excellent and can be purchased for ten cents from some Woolworth Store.

The Stain—Unless the stain is good, the results will not be satisfactory. Alkaline polychrome methylene blue does not keep long. If there is the least doubt about its quality, it should be made up fresh according to directions already published. These have been tested many times, and if carefully followed, they give good stains. It is advisable to use cap anhydrous carbonate and medicinal methylene blue. Other brands may be good but sometimes the substitution of another has led to failure.

Directions for an improved stain are found in the footnote.*

Many lamps have been tested. One which is very inexpensive but quite satisfactory is a 60 watt Mazda lamp with frosted white bulb. Occasionally when the sections are thin or are overstained a stronger lamp is better.

The slides with holes in them can be easily made in a few minutes out of cigar box wood. The illustrations accompanying this paper show what they look like and how they are used. If sufficient care is exercised in cutting the tissue to make the surfaces plane parallel, ordinary slides and covers can be used.

It is very important to keep the razor blade clean and smooth. It should be wiped immediately after using. If the razor is rusted or has clotted blood or bits of dried tissue clinging to it the surface of the tissue will be scratched and the microscopic picture will be less good than it should be for there will be displacement of the surface cells.

Technical Steps—These are ten in number.

1 Selection. If malignancy is to be looked for the pathologist as soon as the tissue reaches him should select by careful inspection palpation, and section, the area or areas he wishes to examine microscopically.

2 Excision. Cut out the area selected bearing in mind the subsequent steps of immobilization and slicing. If possible the block should include an advancing edge of the tumor. A convenient size is 1 by 1 by 0.5 cm. although

Neutralized Polychrome Methylene Blue

This stain keeps well and acts rapidly precisely and differentially on both perfectly fresh unfixed tissue and on formalin fixed tissue. There are five steps in making it: (1) The preparation of stock solutions A, B and C. (2) The titration of Solution A against Solution C. (3) The alkalization of B by means of C. (4) The polychroming of the alkalized B. (5) The neutralization by means of C of alkali already added to B.

The first five steps in detail are as follows:

1 The three stock solutions in neutral distilled water are:

A	12% K ₂ CO ₃ C.P. anhydrous	100 cc
B	1% methylene blue medicinal	1000 cc
C	10% acetic acid, by volume	100 cc

2 Titration. Determine how much of Solution A exactly neutralizes 1 cc. of boiling standard Solution C using phenolphthalein as an indicator. Mark this quantity on Bottle A.

3 Alkalization. Into a 100 cc. graduate place that quantity of A, which is equivalent to 1 cc. of C, add enough of B to make 100 cc. and mix thoroughly.

4 Polychroming. Of the alkalized methylene blue 100 cc. are placed in each of four one ounce bottles. These are stood unstoppered in cold water which is brought to a boil in about 10 minutes. Note the time and remove the bottles one by one 15, 0, 5 and 30 minutes later. Let them cool slowly. The water after reaching the boiling point should be kept boiling while the bottles are in it.

5 Neutralization. To each 5 cc. of the polychrome stain add 0.5 cc. of Solution C. Titration is usually unnecessary and should not be carried out immediately. The four bottles are polychromed differently to permit each worker to make and choose stains which best suit his own taste, light and work.

Two firms in New York City, the National Aniline and Chemical Company, 40 Rector Street and Elmer and Amend, Third Avenue at 18th Street have begun to make the stain here described and each has agreed to submit samples for my approval before marketing it.

smaller or larger blocks may be successfully cut. A small block properly excised is much better than a larger one not so carefully selected.

3 Immobilization. If tissue is to be sliced thin and smoothly, *it should not move while it is being cut*. The block is immobilized by carefully pinning its broadest and flattest surface to the cork plate, using two or more pins, the number depending on the size, shape, and consistency of the tissue. The illustrations will show how the pins are used. The tissue may be further supported by forceps. If the technician is right handed, it is convenient to pin the tissue 2 to 3 cm. above and to the left of the lower right corner of the cork. If the block consists mainly of soft tissue but has a dense capsule or

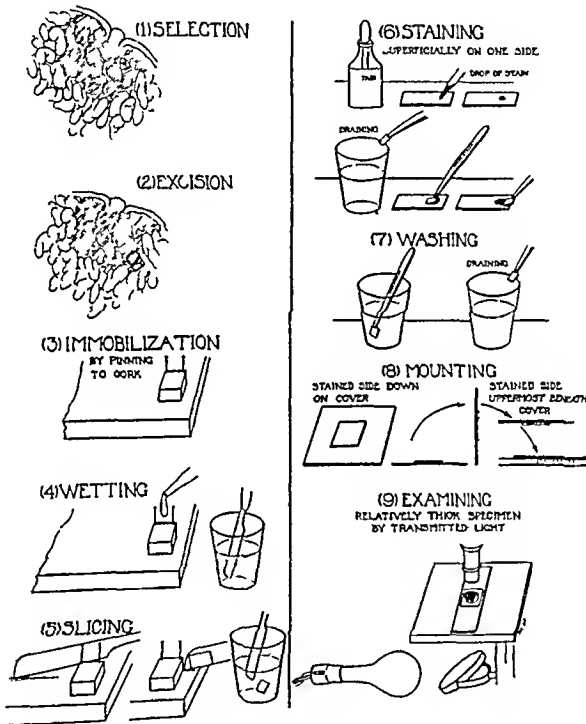


Fig. 2—Nine steps in preparing the tissue for microscopic examination

a tougher part, the latter should be pinned underneath. Otherwise the softer portion may be crushed when an attempt is made to cut through the tissue.

4 Wetting. In order to disturb the tissue cells as little as possible, just before slicing, both the razor and the tissue should be wet with distilled water, clean tap water or physiologic salt solution*. Wetting reduces friction and makes it easier to secure perfect sections.

5 Slicing. The object is to obtain one or more thin, smooth, plane-parallel slices which shall contain the area that one wishes to examine microscopically. Rest the point of the razor on the cork plate about 5 to 6 cm. to

*Theoretically physiologic salt solution is to be preferred but in my experience the tap water in Nashville, Toledo, Chicago, Cleveland, Rochester and other cities has given such excellent results that I usually use tap water or distilled water. The reader should try all three and choose the one that suits him best.

the left of the immobilized wet tissue. The point if properly placed is not raised again until the section is completely severed from the block. Before sectioning the other end of the razor is raised and then lowered over the block so that by sighting over the blade one can see exactly how much tissue is to be cut off. *Do not press the razor into the tissue.* That would crush it. Instead, use it as if it were a very fine saw. Neither raising nor lowering the blade draw it along the cork, using as near as possible the full length of the blade. In this way cut with one stroke completely through the tissue about 1 to 2 mm in front of the supporting pins. If the razor is very sharp its own weight, as it is drawn along, will be almost enough to cause it to cut entirely through tissue of average resistance. The first piece cut off is usually discarded. Without changing the position of the razor in the hand place the point once more on the cork to the left of the immobilized tissue and sighting over the blade to see how thin a piece you can cut draw the wet razor once more through the tissue this time securing a thin plane parallel slice which can be stained at once or deposited in a small vessel containing distilled or tap water. In slicing be careful not to bring the edge of the razor in contact with the pins. With a little practice you will be able to get surprisingly thin sections. But thin sectioning can be overdone. If the sections are too thin they are harder to handle and before staining are best mounted on slides while they are still in water. Moreover sections which in the beginning you are apt to regard as too thick will probably give better results than you anticipate. If the amount of tissue is very small every section cut should be stained and examined.

6 Staining. The stain should be applied superficially to one side only. There are several ways of doing this. The following is one of the best and works well where the tissue is so thin that mounting is difficult. Place one end of a clean slide in the glass of water containing the sections and with forceps bring a thin, smooth flat section in contact with the slide and pull both out of the water at the same time. This usually causes the section to spread out and lie flat on the slide. Without disturbing the section wipe off the excess of water on the slide. With a medicine dropper place on the slide near the section a small drop of good neutral polychrome methylene blue and spread it out thin. With forceps pick up one end of the section and raising it about 1 mm drag it over on top of the stain and move it about gently for approximately 1 to 5 seconds. Carefully avoid getting stain between the prongs of the forceps or on both surfaces of the section. If this latter occurs, discard the section and try again. Unless the section is moved about, it may not stain uniformly, for the weight of the wet section may suffice to press out from beneath it most of the stain. The time required to stain varies with the strength of the stain, the amount of water dragged into it and also with the vividity the cells have for the stain. If the section is not stained heavily enough the first time, it can be restained for a second or so. Thin sections are sometimes best stained by mounting them on a slide as already described then after draining off the excess water turn the slide over so that the section is below. With a fine camel's hair brush wet with the stain, the

ning have no difficulty with razor sections of favorable tissues, such as most breast tumors. But pathologists experienced in the diagnosis of fixed tissue only, should, until they are thoroughly familiar with the appearance of unfixed tissue cells, control all of their razor section diagnoses by the method they know best.

NEW METHOD

In believing this method new I may be in error, although up to the present I have found nothing quite like it in the literature. The newness, however, does not lie in using a razor to section tissue. Before the discovery by Stilling,⁸ Jan 25, 1842, of the advantages of freezing tissue and before the introduction of microtomes by H. D. Schmidt,⁹ Hensen,¹⁰ His,¹¹ (1866), Julius Cohnheim,¹ and others the razor was frequently used for sectioning tissue. The older pathologists knew that unfixed tissue is often soft and difficult to cut. They tried various methods of immobilizing it holding it between their fingers, embedding it in amyloid liver, in shed elder pith, etc. Then they learned that fixed tissue is much easier to cut and they became surprisingly skillful in making razor sections of this. Nevertheless, so far as I am aware, no one of them hit upon the method here suggested of staining thin plane parallel razor sections of unfixed tissue very superficially on one side only, using a rapid vital stain, and examining the sections under glass by transmitted light. This seems to be new.

VALUE OF THE NEW METHOD

The new method has now been tested on over six hundred fresh unfixed tissues diagnosed malignant by the Pathologists of the Mayo Clinic as well as by me. In many cases the tissues were very easily diagnosed. Some tumors were more or less difficult requiring a number of sections to be cut before the diagnosis could be made. A few in the fresh state were practically impossible of diagnosis. Up to the present however apparently no false positives have been given. The number of cases found to be easy increased as the technique improved. The accompanying table shows some of the things learned in examining the first six hundred malignant tissues.

In the table I have marked as more or less difficult all examinations in which I failed to record at the time that I regarded the diagnosis as easy. But my records of the ease or difficulty experienced in examining the first two hundred cases are somewhat incomplete due to interruptions caused by visitors. My statistics showing more or less difficulty in thirty eight of the first hundred and in eighteen of the second hundred are for this reason not quite fair to the method. But I have let them stand as I cannot correct them accurately. Another thing that has reduced the percentage of cases easily diagnosed is the fact that occasionally the lesions were very small and the part that I had for examination may not have contained any malignant areas.

The organ most frequently examined was the lymph node. The easiest was the breast. The most difficult tissues were small friable uterine scrapings and small soft papillomatous carcinomas. These constituted only a small part of the total number of tissues examined.

TABLE SHOWING THE RESULT IN EXAMINING 600 FRESH, UNFIXED TISSUES, GIVING TOTAL NUMBER AND ALSO INDICATING HOW MANY SPECIMENS IN EACH 100 EXAMINED WERE FOUND MORE OR LESS DIFFICULT

	1—100		101—200		201—300		301—400		401—500		501—600		SUMMARY	
	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	1—600 TOTAL	MORE OR LESS HARD
Lymph nodes	22	8	34	8	33	1	25	0	24	1	31	3	169	21
Breasts	23	5	11	0	21	0	15	2	21	0	17	0	109	7
Stomach	10	6	9	3	9	2	16	0	6	0	16	0	66	11
Cecum	4	2	2	0	1	0	4	0	3	0	3	0	17	2
Rectosigmoid	14	10	14	3	15	1	12	0	14	0	10	0	78	14
Miscellaneous	27	7	30	4	21	5	23	1	32	7	23	1	161	25
Total	100	38	100	18	100	9	100	3	100	8	100	4	600	80

A Comparison—For over ten months razor sections and frozen sections of the same tissue have been compared. According to pathologists who have seen both, the razor sections in nearly every instance were faster, and in many cases were said to be better stained and easier to diagnose than the frozen sections.

Dependability—Reportedly microscopic diagnoses have been reported to the operating room by Drs. MacCarty, Broders and Caylor on razor sections before the frozen sections were ready to be examined. Razor sections have been good enough for Dr. MacCarty^{12, 14, 15} to make from them his cytologic diagnosis of malignancy. They have also enabled Dr. Broders^{16, 17} to apply successfully his rules for grading malignancy.

Many doctors visiting the Mayo Clinic in the last few months have seen the new method and have been very enthusiastic about it. The qualities which seemingly have made the strongest appeal are its simplicity, inexpensiveness, rapidity, and dependability.

Tested by Others—Under my direction a number of physicians have tested the technique, and on favorable tissue no one has failed. Most of the doctors made good sections at the first or second attempt.

ACKNOWLEDGMENTS

During the last eleven months I have been privileged to work as a guest in the Department of Surgical Pathology of the Mayo Clinic. Free access to an abundance of perfectly fresh material has been given me and everything possible done to facilitate my work.

Without the splendid spirit of helpful cooperation shown me the work here reported would have been greatly delayed and might never have been done. For the material put at my disposal, for the interest taken in my work and for the aid they have so freely given, I desire here to express to Drs. MacCarty, Broders, Caylor and Owen my very deep appreciation and cordial thanks.

To Drs. Robertson and Mills I also gratefully acknowledge my indebtedness for many opportunities of examining autopsy material.

SUMMARY

The method of tissue examination described in this paper is apparently new, easy, inexpensive and dependable. It is applicable to fresh unfixed tissue and it permits the microscopic study of cells that are seemingly still alive. The technique for fresh tissue can be applied also to formalin fixed tissue. Artefacts are few. The method is so rapid that reliable microscopic diagnoses of tissue can often be given by an experienced pathologist in less than sixty seconds. While it is probably especially useful in the diagnosis of malignancy, it is not limited to this. It should be of value in studying and teaching cytology, histology, and pathology. It seems almost ideal for use in the doctor's office, in the operating room, in hospital laboratories, and in an emergency, in the patient's home.

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DISCUSSION

Dr Wm Carpenter MacCarty—I think I know perhaps as much about this method as anyone except Dr Terry, and the best way to learn it is to go and see his demonstration. I have seen it almost daily for the last eight months and would be perfectly willing to take a bottle of his stain, a couple of pins and a razor, and make almost any diagnosis that I would make by any other method. I do not need the microtome. The microtome has the advantage, however, of making a section which will keep a whole day if you want to study it over a long period. So far as actual recognition of the things which are diagnostic I would as soon have Dr Terry's preparation as any made with a freezing microtome. I would advise you to look into his method, for if you are interested in tissue you cannot afford to miss the opportunity. Dr Terry is the first Professor of Pathology who has seen fit to spend time enough to investigate our fresh tissue methods and diagnosis. You cannot expect anyone to learn tissue work in a few months, and it is going to take brains to see and interpret the findings for practical purposes. Dr Terry's method is of very great value not only to the practical tissue diagnostician but to teachers of pathology and histologic anatomy.

I know that in our clinic no clinician has a better diagnostic efficiency by clinical signs and symptoms than 70 per cent. There is probably not a gross pathologist who can diagnose everything as it comes, in over 80 per cent of cases. In my own experience in a study of 42,000 specimens, gross diagnostic efficiency is not over 80 per cent. It must be remembered that no man can become a great gross diagnostician until he is a microscopic diagnostician. In all cases the pathologist should have access to the patient, as well as all clinical data and surgical findings.

Dr Harold G Palmer—I have been tremendously interested in the matter of frozen sections for quite a number of years, as a matter of fact I did the first frozen section that was ever done in Rhode Island some twenty eight years ago. From what I have learned tonight I feel that I am just in the kindergarten grade. I have been impressed with what Dr Terry has shown today and what he has said and with the remarks that Dr MacCarty has made tonight. This method of methylene blue staining with the fresh tissue brings out tremendous possibilities, it seems to me, but we must consider the fact that we have an entirely different angle from which we are looking at the picture. All of our pathology and histology has been passed on in interpretation of a compound structure, considering the morphology of cells and structures as they are arranged. As I understand from my talk with Dr Terry today, with this method the interpretation is based on the study of only the nucleus and nucleoli of the stained cells. I said to Dr Terry that I was interested, I should be glad to follow this through. I would also make my frozen sections and compare them side by side and study the different cells. I feel that it is a very dangerous procedure to go home and say this is easy, we can do this. You cannot go ahead and interpret soundly without having a great deal of experience. I feel that tissues should be submitted to men of

Dr Terry's experience and be checked up on your findings. Another point which is of very vital importance to my mind is this matter of the technicians working on frozen sections and technicians' interpretation. Under no circumstances should technicians interpret on a tissue or any other laboratory procedure.

Their job is to prepare material for the pathologist to study and when such preparation is completed the technicians' obligation and responsibility terminate immediately.

Dr Terry's rapid method for fresh and fixed tissues is a distinct addition to our laboratory technique. The frozen section with hematoxylin and eosin staining will continue to be a valuable procedure and under no circumstances should it be thrown into the discard.

Dr Philip Hillborn — May I discuss this presentation from a practical view? In the maze of details of technique of the microscopic examination one is apt to forget the value of gross appearances. In observing the trees we lose sight of the forest. In most cases where an immediate diagnosis is necessary during an operation the trained pathologist is able to render correct opinion based on the naked eye picture. The hospital laboratory director should school his sense of sight and palpation so as to be able to recognize malignancy on inspection. I am greatly inclined to maintain the thesis that given adequate experience and schooling all tumors can be diagnosed by the naked eye. This is especially true in neoplasms of the breast. Unfortunately not all of us have the opportunity to see a large number of cases or the faculty of profiting by the observation. It is however, an ideal that we should endeavor to attain. So with all due appreciation of the value of improved technical methods in extemporaneous frozen sections permit me to make a plea for greater attention to gross details.

ACCURACY AND PRECISION IN CLINICAL PATHOLOGY*†

By PHILIP V. WELLS, D.Sc., NEWARK, N. J.

INTRODUCTION

IN ANY discussion of accuracy it would seem most appropriate to begin by emphasizing accuracy of statement, because no one who reads scientific literature can help being confused by the looseness and ambiguity generally associated with expressions concerning precision and accuracy. Such statements as "The measurement is good to 1 per cent. The experimental error in no case can exceed 5 per cent," "The method is reliable to $\frac{1}{2}$ per cent," etc., seem to many authors satisfactory descriptions of accuracy. When one faces the facts however, such expressions are seen to be meaningless, and one wonders if the authors are always aware of this. Perhaps the labor involved in obtaining sufficient data to determine the precision of a particular method let alone a significant estimate of its accuracy is in part responsible for this situation.

This paper will be restricted to an effort to define in a practical way the significance of the terms precision and accuracy as they may be applied to clinical pathologic methods.

ERRORS AND REPRODUCIBILITY

An experimental method may be very precise but quite inaccurate. Precision is evident from the data; accuracy is always in doubt. There is a whole hierarchy of errors, each including those of lower order and accuracies

Laboratory of the Prudential Insurance Company, Newark, N. J.
*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, Washington, D. C., May 1st 14 and 16 1927.

is the net result of them all. For example, a hemacytometer may have inaccurately ruled lines, which would give counts systematically high as long as it is used, but its cement might loosen after some months, thereafter reducing the counts systematically, and these two systematic errors might exactly compensate for a time to give correct results. The precision of the counting chamber, if investigated by repeating counts, would not change, because each count would be high by the same amount before the cement loosened, and low by a fixed amount thereafter. To reveal such inaccuracies, different chambers should be compared with each other on the same sample of blood, or better, the actual dimensions of the chamber should be measured. The precision of the blood count, on the other hand, might depend almost entirely upon the mixing in the pipette, or upon the perfection of the blood sampling.

The fluctuations arising from personal idiosyncrasies of technic are usually fairly small, and can be largely eliminated by repetition of the measurement. To attain accuracy, however, both accidental and systematic errors must be reduced. Accuracy implies that a method be reproducible in its entirety and give absolute results, the same in every hospital laboratory throughout the country from one decade to the next, so that experience can accumulate on a fixed basis in sufficient volume to make scientific progress possible. The reagents, the instruments, and the criterion of measurement must all be reproducible from specification, or else continuity must be maintained by means of permanent standards. These requirements are, of course, to be interpreted reasonably, and an instrument need not be ten times as precise as the normal fluctuations in the samples measured. In pioneer work relative results often suffice, but for clinical interpretation methods should be standardized just as are the units of mass, length, and time.

When a particular method happens to be the only one available, differences in the results are apt to be ascribed entirely to variations in the samples, and a spurious specificity is often in this way attributed to the phenomena under study. Only those variations are significant which are not errors of measurement. How are we to distinguish such errors from real phenomena? As a matter of fact, we can never be certain that a given variation is not an error, but correct statistical methods will help us to estimate the chances in favor of one interpretation as compared with another.

AVERAGE VALUES THE MEDIAN

Many errors arise from causes which are quite apparent. The complex interplay of known factors account for some, while the sources of other errors may be wholly unknown. Whatever the origin of the variations observed in a quantity which theoretically should be constant, they are usually lumped together and ascribed to accidental causes or to "chance." Chance variations cannot be predicted individually. In sufficiently large numbers, however, they are supposed to distribute themselves in a fixed manner on either side of the "true" value which they are assumed to represent. Upon the stability of such distributions of errors depends the actuality of what has been called the "true" value. This is a mean or average value the

choice of which is a matter of convention. Indeed, the choice of characteristic to be observed is quite arbitrary, a fact generally overlooked in statistical literature.

It is customary to use the arithmetic mean obtained by adding all the observations together and dividing by their number. This simple average is regarded by some as theoretically the most fundamental, but might better be regarded as a simple approximation to the median or middle value. The median is defined as a value of such magnitude that it stands between the half of the observations which are less, and the half which are greater. The median was considered the "most advantageous" value by Laplace because it rendered the sum of the deviations (taken without regard to sign) a minimum. The arithmetic mean renders the sum of the squares of the deviations (from it) a minimum. Both are arbitrary. The most important advantage of the median is that *it is the only average which does not change with change of variable*.

In analyzing data it is often found that some function of the variable which was originally observed is related to other quantities more simply than is the original variable. For example the square or logarithm might give a straight line when plotted against some other variable. But the square of the arithmetic mean is not the arithmetic mean of the squares of the observations, and the logarithm of the arithmetic mean is always larger than the arithmetic mean of the logarithms. Even the mode, or most frequent value, suffers from the same difficulty. The logarithm (or any other function) of the median, however, is the median of the logarithms (or any other function). This is because the median is defined by frequencies alone, the magnitude of the variation having no other influence than to place it in one of two classes, above or below the median. For the purposes of interpretation, therefore, the *median* is the best average.

In practice the median is simply and quickly determined. A glance at the figures shows about where they tend to cluster. The values falling in this neighborhood are arranged in order of magnitude, those falling above and below are simply counted. Thus if there are twenty values in all, say six below those in order and nine above, the median falls between the fourth and the fifth values arranged in order. Usually both the fourth and the fifth will be equal, but if they differ some convention must be used to define the median. In most cases a value half way between the middle pair of values gives a good median when the number of observations is even. When the number is odd there is but one middle value. Some statisticians use a "smoothed median" to decrease the fluctuations due to smallness of samples. One can therefore take the arithmetic mean of three or four middle values and thus avoid the overemphasis of the ordinary arithmetic mean on extreme values and yet retain its smoothness even with small samples.

The essential characteristic of an average is its stability, which makes it more useful than any individual observation, because it represents all the data and is not dominated by a few. Now in any case where data vary widely, a few very large values representing perhaps less than 1 per cent of the results, may halve or double the value of the arithmetic mean, but

then effect upon the median is slight. This renders the arithmetic mean practically valueless in distributions of wide variability.¹

Everyone has experienced difficulty in deciding what to do with an extreme when only a few observations are available. If it is included, the arithmetic mean is probably biased toward it, and yet it seems arbitrary to discard the observation entirely. The smoothed median avoids any such bias because the middle values are the only magnitudes involved and the extreme variations merely locate the middle values. It is unlikely that the extreme variations should have as much weight as the middle values, whereas the arithmetic mean gives them the most weight. The smoothed median is, therefore, the most appropriate average for small samples.

THE AVERAGE DEVIATION AS A MEASURE OF VARIATION

The simplest measure of the amount of variation among the observed values is the sum of the deviations from the median (taken without regard to sign) divided by their number. This is usually called the *average deviation*, and abbreviated *ad*. Some statisticians call it the mean error, but others use this expression for the square root of the arithmetic mean of the deviations squared, which is generally called the standard deviation (σ). Squaring the deviations is a mathematical device for eliminating the negative sign. Moreover, the arithmetic mean of the observations is the average which renders the standard deviation (from it) a minimum, just as the average deviation taken from the median is a minimum.

For mathematical statistics the arithmetic mean and standard deviation are more convenient than the median and the average deviation, but in practice the latter are much easier to compute. Logically, the advantages are also with the average deviation, for the standard deviation gives altogether too much weight to extreme deviations, which are often mistakes of the observer, and without significance. With small samples, the difference between the values of the average and standard deviations is of no importance (except to emphasize the insufficiency of the data) so that it is pedantic to perform the extra work of squaring the deviations. To shorten the labor, the *ad* should not be computed from the exact value of the median, but from the nearest round number. It is useless to retain more than two figures in deviations, and one figure is often sufficient.

It is possible to compute the *ad* every time an average is taken, but, of course, it would be a waste of time. Nevertheless, it is impossible to interpret data correctly without an approximate knowledge of the *ad*, for without it errors cannot be distinguished from the phenomena of interest. Therefore, it should be the first duty of the interpreter of data to learn the *ad*'s of the averages used.

The *ad* of a single observation from the mean is merely a measure of the precision of the particular observations included. If these constitute a sufficient sample of the universe under discussion, the *ad* from the mean is also a measure of the accuracy of the data. Unfortunately there is no royal

¹This point is discussed in more detail in section 9 of a paper by P. V. Wells and W. F. Wells Jour. Am. Water Works Assn. 1922 ix 502-27.

word to accuracy. The best we can do is to try to find the possible causes of variation, and to test the possibilities experimentally. Now, every such test means a series of observations from which an *ad* can be computed which is the proper measure of the precision indicated by the test. The systematic errors are shown by comparison of the averages characterizing the conditions in different tests with the same standard. When all sources of error have been eliminated as far as possible and the technique of the method decided upon, *the final accuracy of the method can be expressed by the average deviation of a single observation from the mean of a series which includes all the variations with the same frequency as is met in practice*.

This definition of accuracy assumes a complete knowledge of all the technical details involved in practice but only the widest variations need challenge attention because each deviation is the net result of all the random forces at play which tend to neutralize each other. Indeed it is always found experimentally that large deviations occur much less frequently than small ones, also that the average deviation in one series of observations is about the same as the *ad* in a second set under similar conditions. It is this stability of the average deviation which makes it useful in forecasting the future.

CHANCE VARIATION

Suppose two samples show a difference. Is the difference real or due to experimental error? Such a question poses the fundamental problem of accuracy which is the determination of the chance of its being one or the other. The chance is to be expressed in terms of the average deviation of a single observation from the mean of a series in which every factor except the sample has been allowed to vary at random. If this series were extensive enough, a smooth frequency curve of the deviations could be drawn with the magnitude of the deviation as abscissa and the relative number of deviations of that magnitude as ordinate. This curve would rise rapidly to a maximum at zero deviation then fall again more or less rapidly. On the other hand, if the deviations were plotted one after another in the order observed they would show no regularity but would simply fluctuate above and below the zero line as do winners in games of chance. We cannot predict the next deviation however extensive our experience and so we say it occurs by chance. But the shape of the frequency curve can be predicted with considerable accuracy for if another large number of observations be made and a frequency curve drawn it will be of practically the same shape as the first. It is the reproducibility of the frequency curve of the deviations and the random order of their occurrence that are characteristic of chance variations.

Experience shows that more than one half of the deviations are less, and less than one half are usually greater than the average deviation. There is therefore somewhat less than one chance in two that the next deviation will exceed the *ad* in magnitude. Further there is usually a tendency for the errors to group themselves symmetrically so that the chances of negative and positive deviations of equal magnitude are equal. Two simple negative

exponential functions have been found to represent approximately the frequency distributions in many cases. One is appropriate to the arithmetic mean and standard deviation, while the other is appropriate to the median and the average deviation. The law of the standard deviation involves squares in the exponent, the law of the average deviation involves only the first power. Tchebycheff has deduced a criterion which states that a deviation greater than (T) times the standard deviation (σ) occurs less than once in (T^2) times. While this makes no assumption as to the form of the frequency curve, unfortunately wide deviations usually occur very much less frequently than this upper limit. The chances indicated by these three criteria are shown in Table I. They emphasize caution in asserting that a phenomenon is real without knowing the actual frequency curve.

TABLE I
FREQUENCY OF POSITIVE DEVIATIONS EXCEEDING $T\sigma$

T	TCHEBYCHEFF'S CRITERION LESS THAN —ONE IN	LAW OF STANDARD DEVIATION ONE IN	LAW OF AVERAGE DEVIATION ONE IN
1	2	6	8
2	8	43	34
3	18	743	139
4	32	31500	568
5	50	—	2347

LAW OF THE AVERAGE DEVIATION

Frequency of deviations exceeding $\pm x$ is equal to $\exp(-x/a d)$									
Deviation ($\pm x/a d$)									
1	2	3	4	5	6	7	8	9	10
Frequency one in									
2.7	7.4	20	55	148	403	1096	2985	8130	22222

If all sources of error are properly represented in the average deviation, Tchebycheff's criterion is too conservative, while the normal law probably errs in the opposite direction. According to this law the standard deviation is 1.25 times the average deviation, while the law of the average deviation gives the factor 1.41.

It is customary to state the "probable error" (pe), or median error, without regard to sign instead of the ad or σ , after an average, to indicate its precision. The median error has the same advantages among errors as the median has among averages. The probable error of a single observation is divided by the square root of the number of observations to obtain the probable error of the average. If the normal law holds, the probable error of a single observation (pe) is given by $pe = 0.845 ad = 0.6745\sigma$, while the law of the average deviation gives $pe = 0.693 ad = 0.49\sigma$. One-half the deviations exceed the probable error in absolute magnitude. In view of the uncertainty, however, regarding the actual distribution of the deviations, there is little point in using the probable error. The average deviation of a single observation, with a statement of the number of observations averaged, is sufficient.

The true probable error of the average, that is, the median deviation of a series of averages upon the same sample is seldom as small as the probable error of a single observation divided by the square root of the number of observations, because of systematic errors. There is no doubt, however, that the precision of the average increases with the number of observations included, but the returns of the extra labor diminish very rapidly.

Similarly, the rule that the resultant error is the square root of the sum of the squares of the component errors often fails quantitatively because these errors are not entirely independent of each other, as assumed in deriving the rule. Nevertheless the conclusion that only the major sources of error need be considered is amply justified by experience.

ACCURACY OF THE DUBOSCQ COLORIMETER

To illustrate the above discussion some data are presented indicating the accuracy of measurements with the Duboscq colorimeter. The photometric field of the instrument used had a dividing line which practically disappeared when matched, so that the precision of the color comparison is probably better than that of the average Duboscq. Table II gives the results of a comparison of solutions of cuprimmonium sulphate, the concentration in one cup being always twice that in the other. If this solution obeys Beer's law, the observed depths should be in the same ratio as the dilutions (reciprocal concentrations) and the data show no systematic departure from this dilution law over a 64 fold range in dilution. The last comparison is in error because the solution was too dilute to give enough color. To increase the color density of the blue copper solutions and limit the spectral range a monochromatic red filter was tried but the improvement was not as much as expected.

The average deviation of a single observation is less than 1 per cent so that the average depth ratios should agree still better with the dilution ratios. The actual errors however are more than 3 per cent. Tchebycheff's criterion would indicate that there is less than one chance in fifteen that these departures are experimental errors and yet there is no reason to sus-

TABLE II
DILUTION LAW ON DUBOSCQ COLORIMETER (B AND I MICRO MODEL)
UNIT DILUTION = 5% $\text{CuSO}_4 \cdot 4\text{NH}_4 \cdot \text{H}_2\text{O}$

DILUTIONS COMPARED		OBSERVED DEPTHS				DEPTH RATIOS		DILUTION RATIO	ERROR	
		WHITE		RED		WHITE	RED		WHITE PER CENT	RED PER CENT
LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT					
1	1	10.0	9.9	2.0	3.9	0.99	1.99	1	-1.0	-2.0
1	2	10.0	20.7	2.0	4.7	2.04	1.88	1	3.4	-6.0
4	2	30.0	14.7	10.4	5.0	0.483	0.481	0	-3.4	-3.8
4	8	14.1	30.0	5.0	10.0	2.13	2.00	2	6	0
16	8	30.0	14.8	30.0	14.9	0.493	0.494	0	-1.4	-0.6
16	32	14.6	30.0	14.5	30.0	2.15	2.07	2	7.8	3.7
64	32	30.0	15.4	30.0	16.2	0.513	0.54	0.5	9.6	8.0
64	128	12.2	30.0	12.9	30.0	2.46	2.34	2	23.0	16.7
<div><div>White</div><div>Red</div></div>										
Average error (dilution ratio = 2) = +0.9% -0.2%										
Average error (dilution ratio = 0.5) = -0.7% +1.0%										
Average error without regard to sign = 3.9% 3.5%										
A. d. of single observation = 0.8% 0.7%										

Red Written 1 red filter over eye piece White No filter over eye piece

TABLE III
CONCENTRATION LAW ON B AND L DUBOSCQ COLORIMETER
BENEDICT PICRIC ACID SUGAR TECHNIC
KNOWN GLUCOSE CONCENTRATIONS COMPARED WITH 1 MG STANDARD

CONCENTRATION (MG / C C)	OBSERVED DEPTHS		DEPTH RATIOS		PER CENT ERROR		DIFFERENCE	
	SERIES 1	SERIES 2	SERIES 1	SERIES 2	SERIES 1	SERIES 2		
Standard at 10 mm depth								
0.2	55.0	61.0	0.182	0.164	-9	-18	9	
0.3	40.6	45.0	0.246	0.222	-18	-26	8	
0.4	26.2	25.6	0.383	0.391	-4	-2	-2	
Standard at 20 mm depth								
0.5	47.3	53.6	0.423	0.373	-15	-26	11	
0.75	29.0	30.4	0.690	0.658	-8	-12	4	
1.0	20.0	20.1	1.000	0.995	0	0	0	
Standard at 70 mm depth								
1.5	40.2	51.7	1.74	1.35	16	-10	26	
2	33.4	31.0	2.09	2.26	4	13	-9	
2.5	27.6	25.6	2.54	2.73	2	9	-7	
3	26.6	25.2	2.63	2.78	-12	-7	-5	
4	19.7	18.6	3.55	3.76	-11	-6	-5	
5	17.2	17.6	4.07	3.98	-19	-20	1	
6	13.5	13.8	5.18	5.08	-14	-15	1	
7	12.2	12.4	5.74	5.64	-18	-19	1	
8	10.7	11.0	6.54	6.36	-18	-21	3	
10	9.4	9.7	7.45	7.22	-26	-28	2	
20	5.9	6.6	11.9	10.6	-40	-47	7	
40	5.5	6.4	12.7	10.9	-68	-73	5	
80	5.7	6.0	12.3	11.7	-85	-85	0	
Arithmetic mean error (conc 0.33 mg)					=	-4%	-7%	3
Average error (without regard to sign)					=	±9%	±12%	8
A d single observation					=	0.5%		

pect the accuracy of the dilutions, for every care was taken to make them exact. There is evidently some tendency to bias in color matching which makes the results less accurate than is indicated by the reproducibility of consecutive readings. There is no indication that the illumination (from a Mazda white lamp) was not perfectly balanced, but the fact that the errors tend to be positive when the more concentrated solution is in the left-hand cup, and conversely, shows a slight failure of the instrument to give ratios independent of depth. On the whole, the data indicate that the average error in comparing such solutions in the Duboscq at concentrations above 0.1 per cent will be less than 4 per cent.

This prediction is tested on the data in Table III, comparing the amber colors developed in glucose solutions of known concentrations by the Benedict picric acid technic. Two series of samples are compared, to indicate the reproducibility of the method. The average difference between the two series in the concentration range from one-third to three times the standard is 8 per cent, which is not much less than the average errors, 9 per cent in Series 1, and 12 per cent in Series 2. Since both standard and samples for this test were diluted with particular care from a single stock solution, there is no reason to suspect the concentrations, so that the increase in the average error must be due to systematic error. This is shown by the algebraic signs of the errors themselves. The arithmetic mean difference between two series is 3 per cent, which checks with the prediction from Table II.

TABLE IV

EFFECT OF DILUTION AFTER REACTION ON PICRIC SUGAR TECHNIC
 1 MG STANDARD AT 20 MM DEPTH
 SAMPLE OR STANDARD DILUTED TO BRING DEPTHS BETWEEN 10 AND 30 MM
 COMPARED ON B AND L MICROCOLORIMETER BY A DIFFERENT OBSERVER FROM TABLE III

CONCENTRATION (MG / CC)	DEPTH RATIO	ERROR PER CENT	ERROR (TABLE III)
0.1	0.20	103	
0.2	0.306	13	-14
0.3	0.378	26	-2
0.4	0.47	15	-3
0.5	0.5	10	-20
0.6	0.66	10	
0.7	0.7	4	
0.75	-	-	-10
0.8	0.84	1	
0.9	0.93	3	
1.0	1.02	-	0
2.0	2.05	-12	10
3.0	3.0	-14	
4.0	3.96	-11	-8
5.0	3.7	-17	
10.0	4.0	-0	-20
Average error (without regard to sign) =			±10.0%
(Conc 0.33)			
A d single observation =			1.1%
A d (omitting one observation in 46) =			0.6%
Median deviation =			0.6%

The effect of dilution with water after the reaction to bring the colors within close range when the standard is kept at a depth of 20 mm, is shown in Table IV. This series of concentrations of a glucose solution was made just as in the experiments of Table III, but was compared on the micro Duboseq of Table II. The color matches in these studies were made by three observers: those in Table IV by Dr. Rose, in Table III by Dr. Schattner, and I made those in Table II. The errors are negative for concentrations greater than the standard in both Tables III and IV. When the sample is more dilute than the standard, the errors are negative in Table III but positive in Table IV. If the systematic errors are ascribed to the samples the only difference in the technic is in the process of dilution after the reaction. Such complications, due to changes in the samples measured, illustrate the importance of standards in studying the accuracy of a method. At the bottom of Table IV is a typical example of the tendency of the average deviation to overemphasize extreme errors. A single observation has vitiated the *ad*. Omitting this one reduces the *ad* by about one half to 0.6 per cent. This is evidently the better value, for it agrees exactly with the median deviation. But while both instruments give color comparisons quite up to the best standards of photometry in precision (*ad* $\frac{1}{2}$ per cent) colorimetric methods as a whole may give average errors ten times as great. Within the usual range of concentrations, one third to three times that of the standard, the average error of the Benedict picric acid technic for sugar is not much better than 10 per cent of the concentration.

It is evident from these examples that the accuracy of a method should be expressed in terms of averages. The tendency to mention the range or

extreme error difference is to be deplored. The range is the most unreliable measure possible and seldom has any important significance. In practice, extreme results are eliminated by repeating the test which leads to the average deviation as a measure of precision. Range depends upon the number of observations. As these increase, the more chance there is not only of a wider range, but also of a freak result of no importance, which spoils the range. It is therefore not the range but the average deviation which repetition stabilizes.

SUMMARY

Correct statistical methods help us to estimate the chance that a variation is real and not experimental error, and the only variations which can be considered significant are those which are not errors of measurement.

Preference is given the *median* because it is the only average which does not change with change of variable.

With small samples the arithmetic mean of three or four middle values gives a smoothed median.

When all possible sources of error have been eliminated, the final accuracy can be expressed by the average of a sample typical of all the errors of the method.

The average error, not the range of the errors in any sample, gives the best expression for accuracy.

Data obtained with the Duboseq colorimeter are given to illustrate above.

DISCUSSION

Dr. Herman Shulit—This matter has always appeared to me to be very important. I wonder why doctors do not take the trouble to do it. Dr. Wells pointed out to us the different forms of determining the reliability of that average, the probable errors, standard deviations, and square deviations. We also should consider, before we make any manipulations, exactly how many values we have secured. I have always felt that in using the series of observations, probably the most reliable figures for this purpose would be an average of the middle three. This should be most significant. In biologic phenomena the number of observations made are usually very small compared to the amount that would be necessary for a larger figure.

Dr. Wells (closing)—The smoothed median is important, as Dr. Shulit suggests, and it is popular among economic statisticians. Averaging the three or four middle figures gives the advantages of the median for a small number of observations. I might also point out that it is often an advantage to express differences in terms of percentage, which is more significant than are the differences themselves. In making tabulations I notice that there seems to be a tendency to compare each series of figures and to say that the variations are about the same, when the simple calculation of the average deviation would make the comparison quantitative.

SYSTEMATIC SEARCH FOR PATHOGENIC INTESTINAL ORGANISMS IN DISCHARGES OF HEALTHY AND SICK INDIVIDUALS*

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THE occurrence of mobile colonies which progress through liquid media at a uniform and characteristic rate of speed has been reported in a previous paper.¹ The characteristic rate of progression, taken in conjunction with the reaction of the given organism toward lactose furnishes a criterion for recognizing identity. With this object in view a semifluid sugar² media has been devised for differentiating picked colonies of intestinal organisms. This paper is a report of further experiences with this semifluid 'motility' media which was used for subculture of 2521 additional colonies, picked from 795 Endo plates inoculated with feces of 505 normal and abnormal individuals.

During this tryout of the motility media, an attempt was made to bring about "enrichment" or overgrowth of the pathogenic organisms at the expense of other organisms. This attempt was a failure. It was found, however, that a 25 per cent solution of glycerin in broth would protect pathogenic organisms against overgrowth by others.

A contrivance was devised for this purpose of facilitating the growth of many discrete colonies on plates. This in turn led to a handy method of performing the agglutination test. And so this paper is a report on a method of differentiating the intestinal organisms systematically and with a minimum expenditure of time.

The specimens examined were principally feces and urine submitted by cooks, ward attendants, and other soldiers functioning as foodhandlers in the army. Specimens from some hospital patients were also submitted to test.

As was to be expected colon and other bronzing organisms tended to overgrow the typhoid organism. In the attempt to favor the growth of the latter, various proportions of brilliant green were added to broth and to bile media. A concentration of brilliant green was found which would enable the typhoid organism to overgrow the colon organism but no mixture of brilliant green was ever found which could favor the growth of typhoid paratyphoid and dysentery organisms at the expense of nonpathogenic bacteria. In like manner citrated caffeine was tried in agar plates. This reagent inhibited all organisms except paratyphoid 'B' and therefore was of little value. The attempt to find a selective media was then given up.

Instead another plan was tried according to which reliance was placed on a growth restraining reagent, namely glycerin. It was found that mixed cultures emulsified in 25 per cent of glycerin retained their original relative proportions for several days. By growing and picking a greater number of colonies pathogenic organisms could be detected with a fair degree of facility.

The spray method of inoculating plates can be made to yield under optimum conditions about 1000 colonies per plate of 95 mm diameter, in practice, one-fourth as many is the average. A pipette was devised (Fig 1) which was simple, effective, and easily cleansed. This pipette yields a satisfactory cloud of fine misty spray. Fecal emulsions were filtered through paper before spraying in order to secure a fine dispersion of bacteria and thus promote the formation of pure rather than mixed colonies. From time to time plates were sprayed with pure or mixed stock organisms in order to test the technic. To prevent danger, the spraying was done in a large metal can in which was kept a layer of 5 per cent cresol solution. A fenestrated tin shield was placed on the plate at the time of spraying, to prevent contamination of the edges of the plate. Upon Endo plates inoculated with fecal emul-



Fig 1—Gross appearance of organisms grown for nine hours in semifluid 'motility' medium. All organisms are proceeding toward the left except those in the two lowest tubes. Top tube contains sterile medium. Tube next to the top *Eberthella typhi* shows one mobile colony. All remaining cultures are gas formers. Two mobile colonies proceeding 'tandem-fashion' are seen in each of the following tubes Nos 4 5 7 11 16 17 18 19 20 and 21 (Photo by Signal Corps U S Army)

sions by the spray method, the average number of discrete pickable colonies obtained was about 225 per plate. The bronzed colonies outnumbered the nonbronzed in the ratio of about five to one. Levine's phosphate Endo containing no meat extract was used. This media is quite delicate, and gives picturesque results, especially when it is twenty-four hours old at the time of inoculation. Harris's formula was found highly satisfactory in making the media.

After twenty-four hours, colonies were picked to semifluid motility media containing lactose and saccharose. This media when contained in slender tubes as pictured in accompanying illustrations (Figs 2 and 3) reveals

TABLE I
SPEED OF PROGRESSION, FERMENTING POWER AND AGGLUTINABILITY OF INTESTINAL ORGANISMS

Progression	GAS	NO GAS	TESTED FOR AGGLUTINATION	
			AGGLUTINABILITY	POSITIVE
Less than 1 inch	22	318	Records incomplete	None
" 1 to 4 inches	801	69	69	20
" more than 4 inches	1048	28	28	2
TOTAL	2114	407		22
(101 colonies failed to grow)				

motility, speed of progression and capacity to form gas from the contained sugars. It was found that of 2521 colonies picked, including typhoid colonies, 97 were motile and failed to produce gas thus suggesting the typhoid and paratyphoid organisms. These suspicious organisms were all tried for agglu-

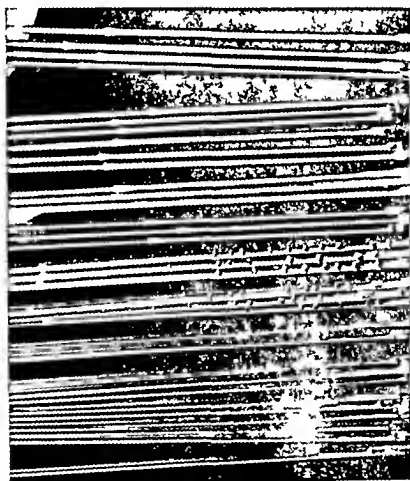


Fig. 2.—Stock organisms grown in motility media for nine hours and proceeding from right to left. Beginning at the top. Tubes 1 to 4 contain *Eberthella typhi*. Tubes 5 to 10 *Salmonella paratyphi*. Tubes 11 to 16 *Salmonella Schottmuelleri*. Tubes 17 to — a gas former probably *Escherichia coli*. All eleven remaining tubes below contain nonmotile organisms. *Eberthella paratyphenteriae* or species of *staphylococcus*. All are inoculated to a depth of one inch in the tube. Nonmotile organisms have not progressed. Each tube containing motile organisms shows a hazy band at the most advanced (farthest left) portion of growth. (Photo by Signal Corps U. S. Army.)

tinability by the method and with the results described below. Table I shows the classification of the organisms subcultured with special reference to their motility, their capacity to form gas in the media employed, and their agglutinability. The motility media indicated whether a given colony should be tried against immune serum of the nonmotile group (dysentery) or against that of the motile group (typhoid and paratyphoid). Table I shows that

about 15 per cent of all colonies grown in motility media required tests for agglutinability, but the bulk of these were nonmotile and, hence, required to be tried only against the antidyentery immune sera

This relatively high number of subcultures requiring agglutination led to a method of agglutination by inoculating the organism into a broth containing a small proportion of homologous or polyvalent immune serum

Numerous authors have reported growth of bacteria in homologous immune sera. Stuyker⁴ enumerates a dozen authors who have observed the behavior of various organisms principally those of the intestinal group under such conditions, and some observers report a loss of agglutinability by typhoid bacilli so grown. Stuyker grew pneumococci in broth containing homologous immune serum, and found that prompt agglutination occurred when pneumococci were subcultured in immune serum for the first time.

The following experiments served as a basis for the agglutination technique described below.

It was found that typhoid organisms inoculated into plain broth containing 0.3 per cent antityphoid immune serum (of a titer of 1:3200) would grow in agglutinated form, showing as fine white granules suspended in practically clear broth. Inoculated into one end of a glass tube $\frac{1}{8}$ inch by 36

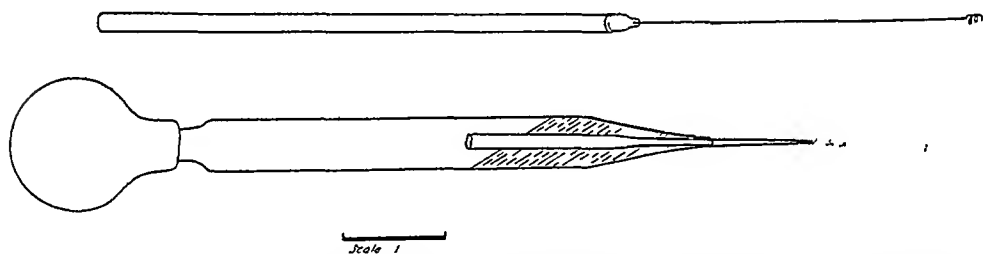


Fig. 3.—Spraying pipette consisting of rubber bulb, large outer tube and small inner tube. Inner tube ends at point where it touches outer tube. Large tube extends on to the end. Fluid level indicates angle in which pipette is held during use. Above spiral wire for introducing immune serum into broth tubes.

inches filled with the immune serum broth, the typhoid organisms continued to progress for several months at the rate of about one inch a week. They were grown in the horizontal position at room temperature. The agglutinated masses could not be broken up by agitating the tube. If a similar tube were filled throughout one-half its extent with plain broth and throughout the remaining half with the immune serum broth as described, the organisms introduced would progress several inches a day (more rapidly than in motility media) while traversing the plain broth, but when encountering the immune serum would form the white granules mentioned above and would slow down their pace. The gross appearance of the organisms underwent a regular and definite change at or near the point where they first encountered the immune broth. In the plain broth they left behind them a narrow streak. As they encountered the immune broth they formed a broader granular streak. The granulations constituting a positive agglutination test can be seen at the end of twenty-four hours in favorable instances, but they are larger and more striking after forty-eight hours. One strain of typhoid organisms was encoun-

tered which did not agglutinate satisfactorily by this technic, namely, the "Rawlin's" strain. Once formed, the agglutinated masses persisted for a year and then gradually disintegrated. Cultures in broth without immune serum remained unagglutinated for two years.

It was found possible to mix the sera corresponding to the three organisms constituting the typhoid, paratyphoid group without losing group specificity. The method of agglutination adopted for routine use was as follows. The mixed "TAB" serum (consisting of antityphoid, antipara A, and antipara B sera) was kept on ice and just before use was added to the broth contained in slender tubes. For transferring and mixing the droplet of serum, a nichrome wire was wound into three spiral turns at the tip, since by actual trial this yielded a droplet of serum which was effective in the tubes used (Fig. 1). It was found difficult to get a satisfactory technic for tubing the immune serum broth until the nichrome spiral was invented. Finally the tubes were inoculated with the organism to be tested. These tubes were about $\frac{1}{8}$ inch by 10 inches in size. The serum was introduced and mixed by the nichrome spiral to a depth of four inches. The inoculum was thrust in to a depth of six inches. The tubes were then incubated at 37° C. for twenty-four hours. Known typhoid or paratyphoid organisms gave typical agglutination as described above. One per cent. xylene was added to the broth used in these agglutination tubes. In positive cases paratyphoid "B" organisms produced gas while the typhoid and paratyphoid "A" organisms formed no gas. It was borne in mind that the differentiation of the pathogenic organisms one from another is relatively simple, while the first separation of the pathogenic from the non-pathogenic organisms is difficult. It was found that the agglutination method was rapid, it enabled us to test each suspicious colony against all three of the motile pathogenic organisms simultaneously. The possibility of infection from the living organisms was reduced to a minimum and the tube was of value because it could be preserved as a record and compared with the others. Apparently, organisms which grow in clumps stand out more clearly than organisms which form clumps after having come into existence in dispersed form. It is to be noted that when immune serum is added to one end of a slender tube which is then inoculated as described the organism comes in contact with virtually all possible dilutions of serum.

A mixed dysentery serum was satisfactory against all but the Shiga organism. These tests, however, were not carried on as consistently as in the case of the typhoid paratyphoid group and a high titer antidyserentery serum of Shiga type could not be obtained.

Practically no bacillus carriers were found. One colony was found which gave the ordinary tests characteristic of the paratyphoid A organism, but repeated examinations failed to reveal any further colonies of this organism. Likewise, one colony gave the ordinary tests characteristic of the paratyphoid "B" organism, but it could not be found again. No typhoid organisms were found except in two cases of almost typical typhoid fever. These two cases furnished 15 plates bearing 9000 colonies of which 844 were regarded as

suspicious Of the suspicious colonies, 132 were subcultured in motility tubes and 53 appeared suspicious Of the 53 tested for agglutinability, 20 were positively agglutinated

For encouraging the study, thanks are due Col E R Schmeier, MC, Commanding Officer, Tripler General Hospital For routine assistance, acknowledgments are due Privates First Class Victor E Del Vecchio and Thomas J Costie, Medical Department, United States Army

SUMMARY AND CONCLUSIONS

1 A serviceable pipette has been devised and described, which is suitable for inoculating plates by spray method, thus yielding many discrete colonies

2 The behavior of an organism when grown in motility media determines whether it should be tried against agglutinating serum of the motile group (typhoid and paratyphoid) or against that of the nonmotile group (dysentery), or whether test for agglutinability may safely be omitted

3 A safe and handy method of performing the agglutination test is described This test yields white granules which preserve their form for months

4 No carriers were found among the 505 soldiers examined A single colony of each of the paratyphoids was seen, but these failed of confirmation on further, oft repeated examinations

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SIMPLIFICATION OF THE TECHNIC FOR THE WASSERMANN TEST

BY LEON H CORNWALL, M D, DESIDERIUS GROSZBERG, M D, AND BLANCHE C TAYLOR, NEW YORK CITY

A SEMIAUTOMATIC distributing apparatus designed to facilitate the operation of pipetting was described by Cornwall and Schmitt in 1922¹ Since that time it has been in constant use in the Pathologic Laboratory of the New York City Hospital, and as a time saver it has earned for itself the respect of our laboratory staff

It occurred to one of us (D G) that further conservation of time could be realized in the performance of the Wassermann test by making three preliminary mixtures of the ingredients and adding more than one ingredient at a time The question immediately arose concerning the desirability of mixing antigen and complement and then adding this to blood serum Data were available concerning the effect of adding serum to a mixture of complement and antigen after the latter had stood for a period of one-half hour Kolmer's² experiments on this point showed that there was a slight increase in

the fixation of complement when this method was compared with the successive addition of serum, antigen and complement. In Kolmer's experiment with eighteen syphilitic sera tested in five doses, there was no difference in the fixation of complement in seven sera. In three sera there was very slightly more fixation with the method of successive addition and in eight sera there was slightly more fixation with the method in which antigen and complement was allowed to stand for one half hour before the addition of serum.

For our purposes we did not contemplate allowing the mixture of antigen and complement to stand longer than the time that would be required to add it to the blood sera by means of the 'distributor'. In view of the fact that, by means of this apparatus, the mixture could be added to sixty tubes a minute or to three hundred tubes in five minutes, we felt certain that this technical alteration would not modify our results to any appreciable extent.

In the same experiment Kolmer's results also indicate a slight increase in the fixation of complement when serum and antigen are allowed to be in contact for one half hour before the addition of complement. With the method of pipetting by hand we could easily confirm the fact that with our usual number of seventy five to one hundred tests the interval during which the complement and antigen were in contact before the addition of the mixture to the sera was much less than the interval during which sera and antigen were in contact before the addition of complement by our usual technique.

Before utilizing the new method as a routine procedure however we compared 416 sera with the old and the new technique. Among this number there were differences in twelve cases the modified technique being more sensitive. An investigation of those twelve divergences revealed that eight sera from cases of known syphilis gave slightly stronger reactions by the new method. Three sera gave negative reactions with the old technique and two plus fixations with the new. In the case of two of these there were histories of miscarriages, and in the third syphilis was denied. In one case no history was obtainable.

The routine technique for the Wassermann test that was used in the Pathologic Laboratory of the New York City Hospital before the modification described herein accords with a description published several years ago¹ with the exception of a few minor modifications.

Volume—This is 10 c.c. or one fifth of the original Wassermann Citron amount.

Antigens—These consist of a cholesterinized and crude alcoholic extract. The cholesterinized antigen is employed in from five to ten units but never in excess of one fifth of the anticomplementary dose. The alcoholic antigen is employed in two or more units but never in excess of one half of the anticomplementary dose.

Incubation—Fifty minutes at 38° C. for cholesterinized antigen and four hours at from 0 to 8° C. for crude alcoholic extract.

Hemolytic System—Sheep cells 5 per cent and antish sheep rabbit immune serum. Cells are sensitized by mixing withamboceptor and allowing to remain at 38° C. for one half hour before use.

Hemolytic Titration—Complement is titrated against two units ofamboceptor, and two units of complement are used in the test.

Two sets of reactions of three tubes each are set up one for the water bath and one for the ice box.

This "set up" applies to both cholesterinized and crude alcoholic antigen sets

The ingredients with the exception of blood serum were pipetted formerly in the order shown in the "set up" The serum was pipetted in the usual manner The antigen, complement, and saline solution were added

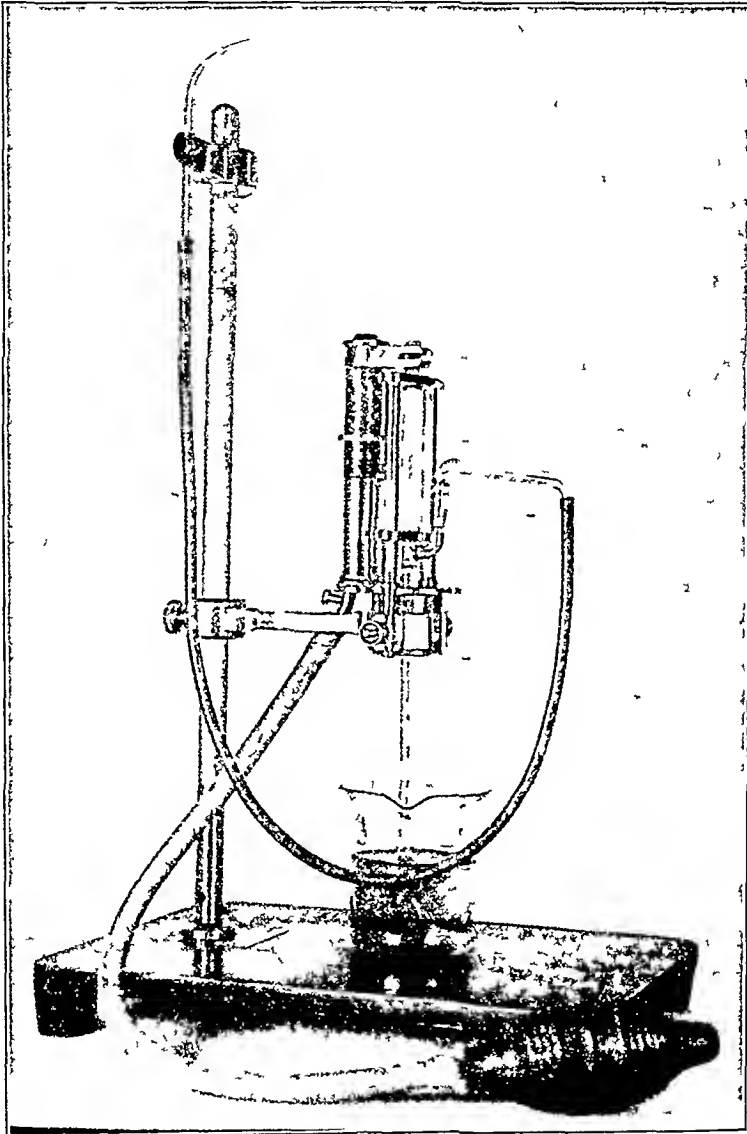


Fig 1—Semiautomatic distributor

separately with the distributor (Fig 1) After preliminary incubation of serum, antigen, and complement, the sensitized cell-antibody mixture was added

The following description will indicate the manner in which the distributor was used formerly It was first adjusted to deliver 0.1 cc, and with this adjustment the crude alcoholic antigen was distributed to the set for the

TABLE I
SIMPLE SET UP FOR BLOOD

	I cc	II cc	III cc
Serum	0.04	0.02	0.04
Antigen dilution	0.2	0.1	0.0
Saline solution	0.16	0.28	0.36
Complement dilution	0.2	0.2	0.2
Amboceptor dilution	0.4	0.4	0.4
Sheep cells 5%			
	1.0	1.0	1.0

ice box. Two deliveries were made to each first tube (0.2 cc) and one to each second tube (0.1 cc). The distributor was washed with saline solution and the cholesterolized antigen was distributed in the same manner to the water bath set. The adjustment was then changed to 0.12 cc and after washing out the distributor, this amount of saline solution was distributed to the second tubes. It was then changed to 0.16 cc, and this amount of saline solution was distributed to all tubes. It was changed again to 0.2 cc and this amount of saline solution was distributed to the third tubes. This

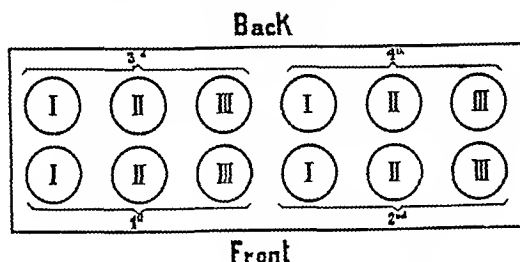


Fig. 2—The set up of on rack carrying four tests

gave a distribution of saline solution as follows: first tube 0.16 cc, second tube 0.28 cc, and third tube 0.36 cc. Using the same adjustment complement was added to all tubes. The distributor was again washed with saline solution, and the adjustment was then changed to 0.4 cc. After preliminary incubation sensitized cell amboceptor mixture was added to all tubes.

This procedure required eight pipetting operations as follows: (1) serum, (2) alcoholic antigen, (3) cholesterolized antigen, (4, 5, and 6) saline solution additions, (7) complement and (8) sensitized cell amboceptor mixture. The distributor was washed three times: first after the addition of crude alcoholic antigen, second after the addition of cholesterolized antigen, and third after the addition of complement.

TECHNICAL MODIFICATIONS

We shall now describe a modification in the technical procedures concerned with the distribution of the several ingredients which economizes time and, in our opinion, conduces to precision.

ARRANGEMENT OF TUBES

The three tubes for each antigen set are arranged in a row. We use racks that have two rows of six tubes each. Both front and back rows are used. Each rack, therefore, serves for four tests (Fig. 2). The racks are arranged on the table one behind the other.

SERUM

A set of empty tubes is arranged and serially numbered corresponding to the tubes containing the sera to be tested. Into each tube 20 c.c. of saline solution are pipetted and also 0.5 c.c. of inactivated serum. The contents are thoroughly mixed, and the pipettes are left in the tubes after the addition of serum. Two-tenths c.c. of the diluted serum is added to the first and third tubes and 0.1 c.c. to the second tubes.

When the amount of serum is insufficient to secure 0.5 c.c. for the dilution, smaller amounts, of course, may be used. The object of diluting the serum is to obviate the necessity of pipetting small amounts, thus diminishing the possibility of small but uncontrollable errors. This feature is in accord with that utilized by Kolmer.⁴

Next, three mixtures of antigen, complement, and saline solution are prepared, the total quantity of each depending upon the number of reactions that are to be performed.

PRELIMINARY MIXTURES

Mixture No. 1 consists of

Antigen	(diluted to the proper titer)	1 part
Complement	(diluted to the proper titer)	1 part

Mixture No. 2 consists of

Antigen	(diluted to the proper titer)	1 part
Complement	(diluted to the proper titer)	2 parts
Saline solution		2 parts

Mixture No. 3 consists of

Complement	(diluted to the proper titer)	1 part
Saline solution		1 part

Mixture No. 1 containing cholesterinized antigen is added to the first tubes of the water-bath set in the amount of 0.4 c.c., and the mixture containing crude alcoholic antigen is added to the first tubes of the ice box set.

Mixture No. 2 containing crude alcoholic extract is added to the second tubes of the ice box set in the amount of 0.5 c.c. The mixture containing cholesterinized antigen is added to the second tubes of the water-bath set.

Mixture No. 3 is added to the third tubes of both sets (water-bath and ice box) in the amount of 0.4 c.c.

The above additions are made with the distributor in the following manner. It is first adjusted to deliver 0.4 c.c., and Mixture No. 3, consisting of complement and saline solution, is added to all third tubes. The distributor does not require washing. Four-tenths c.c. of Mixture No. 1 is then added to the first tubes of the ice box set. The adjustment is changed to deliver 0.5

cc, and Mixture No 2 is added to the second tubes of the ice box and water bath sets respectively. The distributor is again readjusted to deliver 0.4 cc, and Mixture No 1 is added to the first tubes of the water bath set. Thorough washing of the distributor is now necessary before the final addition consisting of the sensitized cell amboceptor mixture (0.4 cc).

The results of these procedures are shown in Table II.

A few simple calculations enable one to prepare a list showing the amounts of the various ingredients that are required for any given number of reactions.

TABLE II

	TUBE I	TUBE II	TUBE III
	CC	CC	CC
Serum diluted 1/5	0.2	0.1	0.2
Antigen dilution	0.2	0.1	0.0
Complement dilution	0.2	0.2	0.2
Saline solution	0.0	0.2	0.2
Sensitized cell amboceptor mixture	0.4	0.4	0.4

TABLE III

SHOWING QUANTITIES OF CONSTITUENTS IN MIXTURES NOS 1, 2 AND 3 THAT ARE REQUIRED FOR FIVE TO ONE HUNDRED TESTS

MIXTURE NO 1 0.4 CC IN FIRST TUBES							
Tests	No	5	10	20	40	80	100
Antigen	cc	1	2	4	8	16	20
Complement	cc	1	2	4	8	16	20

MIXTURE NO 2 0.5 CC IN SECOND TUBES							
Tests	No	5	10	20	40	80	100
Antigen	cc	0.5	1	2	4	8	10
Complement	cc	1.0	2	4	8	16	20
Saline solution	cc	1.0	2	4	8	16	20

MIXTURE NO 3 0.4 CC IN THIRD TUBES							
Tests	No	5	10	20	40	80	100
Complement	cc	2	4	8	16	32	40
Saline solution	cc	2	4	8	16	32	40

The following is a brief resume of the technical operations that have been described above:

1. Prepare a set of tubes corresponding to the number of sera and place 2.0 cc of normal saline solution in each tube.

2. Add 0.5 cc of corresponding inactivated serum to each tube. Mix thoroughly and leave the pipettes in the tubes.

3. Pipette 0.2 cc of the diluted serum into Tubes I and III and 0.1 cc into Tubes II.

4. Prepare Mixtures Nos 1, 2 and 3 for Tubes I, II and III respectively (Table III).

5. Pipette 0.4 cc of Mixture No 3 into all Tubes III.

6 Pipette 0.4 cc of Mixture No 1 (crude alcoholic antigen) into Tubes I of the ice box set

7 Pipette 0.5 cc of Mixture No 2 (crude alcoholic antigen) into Tubes II of the ice box set

8 Pipette 0.5 cc of Mixture No 2 (cholesterinized antigen) into Tubes II of water-bath set

9 Pipette 0.4 cc of Mixture No 1 (cholesterinized antigen) into Tubes I of water-bath set

10 Incubate the tubes of the cholesterinized antigen set in the water-bath at 38° C for fifty minutes and those of the crude alcoholic antigen set in the ice box for four hours at from 0° to 8° C

11 After incubation for four hours, the alcoholic set is removed from the ice box and placed in the thermostat at 38° C for one-half hour, at the end of which period sensitized cell-amboceptor mixture in the amount of 0.4 cc is added to all tubes with the distributor. At the end of the fifty-minute incubation period for the cholesterin set, 0.4 cc of the sensitized cell-amboceptor mixture is added in a similar manner.

The number of pipetting operations by this method for each test tube is three viz, serum, Mixtures Nos 1, 2, or 3, and sensitized cell-amboceptor mixture. The economy of time is obvious. The maximum time economy, however, results from the use of the automatic distributor. Only three adjustments of the instrument are used, namely, 0.4 cc, 0.5 cc, and 0.4 cc, whereas formerly five adjustments were necessary, viz, 0.1 cc, 0.12 cc, 0.16 cc, 0.2 cc, and 0.4 cc. The apparatus requires only one washing from beginning to end, whereas formerly three washings were required.

TECHNIC FOR SPINAL FLUIDS

The time saving feature is equally applicable to spinal fluid Wassermanns. Our routine technic for this consists in testing the fixation capacity of all fluids in three amounts, viz, 0.4 cc, 0.2 cc, and 0.1 cc. These amounts are the equivalents of 20 cc, 10 cc, and 05 cc with the original Wassermann-Citation technic, and in order to avoid confusion, our reports are translated into terms of the original amounts. In cases that are positive in 0.5 cc, a further titration is made with smaller amounts (0.08, 0.04, and 0.02).

TABLE IV
SAMPLE SET UP FOR SPINAL FLUID

		TUBE I	TUBE II	TUBE III
Back Row	Spinal fluid	0.4	0.2	0.1
	Antigen dilution	0.0	0.0	0.0
	Saline solution	0.0	0.2	0.3
	Complement dilution	0.2	0.2	0.2
	Sensitized cell amboceptor mixture	0.4	0.4	0.4
Front Row	Spinal fluid	0.4	0.2	0.1
	Antigen dilution	0.2	0.2	0.2
	Saline solution	0.0	0.0	0.1
	Complement dilution	0.2	0.2	0.2
	Sensitized cell amboceptor mixture	0.4	0.4	0.4

The "set up" for each spinal fluid consists of a front and back row of three tubes each. The back row tubes contain no antigen and are controls, each containing the same amount of spinal fluid as the corresponding front tube.

1 The spinal fluid is pipetted, 0.4 cc, and 0.1 cc, into Tubes I, II, and III respectively of front and back rows.

2 Complement (0.2 cc) is pipetted into all back row tubes.

3 Mixture No. 1 consisting of equal parts of complement and antigen is pipetted into all front row tubes in the amount of 0.4 cc.

4 One tenth cc of saline solution is added to Tubes III of the front row.

5 Two tenths cc of saline solution is added to Tubes II of the back row.

6 Three tenths cc of saline solution is added to Tubes I of the back row.

7 After incubation 0.4 cc of the sensitized cell ambocyte mixture is added to all tubes of the front and back rows.

SUMMARY

The method described reduces the pipetting operations for the Wassermann test to three for each test tube.

Blood serum is diluted with saline solution as in the Kolmer technique and the mixture is added to each tube.

Complement, antigen and saline solution are combined in such a manner that all of them are added at one operation by means of a semiautomatic pipetting device.

Sensitized cell ambocyte mixture is added in a similar manner.

The semiautomatic pipetting apparatus accomplishes a conservation of time.

All ingredients except blood serum are added with the same measuring pipette, thus enhancing precision.

These technical modifications have three manifest advantages: simplicity, rapidity, and precision.

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55 EAST SEVENTY-SIXTH STREET

DISCUSSION

Dr. H. E. Brown—I would like to ask if there were any comparative results of this test with the other tests?

Dr. Grosberg (closing)—Before we used our new method, 416 tests were compared. Twelve divergences resulted. The new method was more sensitive and gave stronger reactions. In three we had positive reactions where the old method showed negative results and we found that the patients had chronic syphilis. The comparison was favorable as far as the new method is concerned.

AN ANTIGEN FOR USE IN SERUM TESTS FOR SYPHILIS*

BY B S KLINE, M D, CLEVELAND, OHIO

ANTIGEN emulsions, quantitatively prepared from an acetone-insoluble lipid wax obtained within a few days as described below, give better results in the microscopic slide precipitation test for syphilis than Kahn antigen dilutions and give good results in the Wassermann test

The antigenic property of acetone-insoluble lipoids in serum tests for syphilis was first reported in December, 1907, by Porges¹ and by Levaditi and Yamanouchi² Since then the work of Nogouchi,³ Brownning, Cruikshank, and M'Kenzie,⁴ Kolmer,⁵ and others, has established the reliability of acetone-insoluble lipid solution as an antigen in the Wassermann test The wax recommended by these workers requires weeks for its isolation A method of obtaining an acetone-insoluble lipid wax, thoroughly satisfactory in serum tests for syphilis, within a few days, is described below

PREPARATION OF THE ANTIGEN

Briefly stated the procedures for the preparation of the antigen are as follows

Shake a mixture of 200 gm of beef heart powder, and 1,000 cc of absolute ethyl alcohol (99+ per cent) vigorously for from twenty to thirty minutes

Filter

Chill the filtrate at 8° to 10° C for from fifteen to twenty four hours

Concentrate the filtrate to 1/20 of the original volume at 45° to 50° C

Pour into 1,000 cc of acetone C P at 35° C

Leave at room temperature for thirty minutes

Decant acetone

Spread wax in thin layer on filter paper, and allow acetone to evaporate

Add 20 cc of absolute ethyl alcohol (99+ per cent) to each gram of wax

Shake for several minutes

Place at 56° C for thirty minutes, shake for several minutes, then place at 8° to 10° C for forty five minutes

Filter

Evaporate filtrate to wax (antigen wax) at 45° to 50° C

Add 10 cc of absolute ethyl alcohol (99+ per cent) to each gram of wax

Shake for several minutes

Place at 56° C for thirty minutes, shake for several minutes, then place at 8° to 10° C for forty five minutes

Filter The filtrate is the antigen

The details of the preparation of the antigen are as follows

The two hundred grams of dried heart powder (Difco) is placed in a two liter Erlenmeyer flask

One liter of absolute ethyl alcohol (99+ per cent) is added

*From the Laboratory Department Mount Sinai Hospital

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After the flask is stoppered with a cork covered with tin foil, it is shaken vigorously by hand or in a shaking machine for from twenty to thirty minutes (This short extraction removes almost all of the desired antigenic substance in the powder)

The extract is filtered into a liter cylinder through good grade filter paper of medium texture (Schleicher and Schull No 597, 32 cm)

During filtration, the mixture is stirred with a wooden tongue depressor and toward the end, pressed with the cork until the powder is quite dry

The extract (about 775 cc) is placed in the refrigerator at 8 to 10 C for from fifteen to twenty four hours

During this time, a fairly heavy white precipitate settles out. This is filtered off and the filtrate in a large evaporating dish is concentrated about twenty times (to about 35 cc) on a water bath or by an air heater (bathroom heater) at 45 to 50 C determined by a thermometer bulb within the extract (If the extract is concentrated much further the resultant wax is not quite as satisfactory)

The concentrated extract is poured into 1,000 cc of acetone C P (Coleman and Bell) at 35 C in a liter cylinder (It is important that the acetone be at this temperature. If it is much lower than this, an unsatisfactory sticky wax resembling that of a plain alcoholic extract results. If the acetone is too warm at 42 C or more, a considerable amount of the lecithin remains in solution. To raise its temperature the acetone is placed in an incubator at 37 C for at least thirty minutes. A liter cylinder is warmed for use at the same time)

When properly prepared, an abundant flaky, light brown precipitate quickly forms and settles. That adherent to the sides of the cylinder is dislodged with a long glass rod about fifteen minutes later

To be sure of complete precipitation the cylinder is allowed to stand at room temperature an additional fifteen minutes after which the acetone is carefully decanted. The soft brown wax is removed with a glass rod and spread thinly on filter paper of close texture, and placed in a current of warm air (45 to 50 C) for about thirty minutes or allowed to stand at room temperature until the odor of acetone is no longer detectable

The wax is then weighed (about 4 gm is the average yield) and 20 cc of absolute ethyl alcohol (99+ per cent) is added to each gram of wax in a glass stoppered bottle

After a few minutes shaking the bottle is placed in a paraffin oven (at 56 C) for thirty minutes to dissolve as much of the wax as possible (the wax is not completely soluble in alcohol)

On removing the bottle from the oven it is shaken for a few minutes and then placed in the refrigerator at 8 to 10 C for forty five minutes

It is then filtered and the filtrate is evaporated down at 45 to 50 C (water bath or air heater) resulting in a soft brown wax (antigen wax). The wax is weighed and to each gram, in a glass stoppered bottle, 10 cc of absolute ethyl alcohol (99+ per cent) is added. After the bottle is shaken for a few minutes it is placed at 35 C for thirty minutes and then shaken a few minutes. (The wax although obtained from a clear alcoholic solution is again incompletely soluble in alcohol)

The slightly turbid solution is placed at 8 to 10 C for about an hour and then filtered. The resultant clear filtrate is the antigen and contains about 875 per cent of the alcohol treated acetone insoluble wax (This wax if repeatedly treated with alcohol or precipitated with acetone is still incompletely soluble in alcohol)

The antigen is kept at room temperature

FORMULAS OF ANTIGEN EMULSIONS FOR THE MICROSCOPIC SLIDE PRECIPITATION TEST

It has been found advisable to use two antigen emulsions in the precipitation test for syphilis one, very sensitive giving results in agreement with Wassermann tests done with cholesterinized antigen the other, less sensitive giving results in agreement with Wassermann tests done with noncholesterinized antigen

1 VERY SENSITIVE ANTIGEN EMULSION

0.85 cc Distilled water
 1.25 cc of 1% Cholesterin (Merck) in absolute ethyl alcohol (99+%) (Prepared in about forty five minutes by placing in a paraffin oven at 56° C and shaking a few minutes at fifteen minute intervals)
 0.1 cc Antigen
 2.2 cc of 0.85% Sodium chloride (C P Merck) solution

2 SENSITIVE ANTIGEN EMULSION

0.85 cc Distilled water
 0.75 cc of 1% Cholesterin (Merck) in absolute ethyl alcohol (99+%)
 0.1 cc Antigen
 2.7 cc of 0.85% Sodium chloride (C P Merck) solution

These emulsions are prepared as follows

Into a one ounce bottle 0.85 cc of distilled water is pipetted

The bottle is held at an angle and the 1 per cent cholesterin (Merck) in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the bottle

The bottle is gently rotated from the neck for from fifteen to twenty seconds

The bottle is held at an angle again and 0.1 cc of antigen is pipetted against the side from a 0.2 cc pipette (graduated in thousandths)

The bottle is promptly stoppered with a cork covered with tin foil and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for thirty seconds

Lastly, the 0.85 per cent sodium chloride (C P Merck) solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for thirty seconds

The emulsions, showing numerous very fine particles at a magnification of 75 times, are now ready for use in the microscopic slide precipitation test for syphilis. They are thoroughly satisfactory immediately after preparation or at any time within three days (kept at room temperature). One drop from a fine capillary pipette equal to about 0.0075 cc is the amount added to 0.05 cc of serum

FORMULAS OF ANTIGEN EMULSIONS FOR THE WASSERMANN TEST

1 CHOLESTERINIZED ANTIGEN EMULSION

1 part very sensitive antigen emulsion (see above)
 29 parts distilled water

2 NONCHOLESTERINIZED ANTIGEN EMULSION

0.1 cc antigen
 5 cc distilled water

These emulsions are prepared by pipetting the slide test emulsion or antigen first and then adding the distilled water gradually, at the same time shaking the bottle

DISCUSSION OF ANTIGEN AND FORMULAS

If the alcoholic extract of heart powder is concentrated shortly after its preparation, a wax is obtained that usually contains an impurity which appears as small colorless granular plates in the slide test, and so interferes with the reading. As indicated above, the extract is freed of this impurity by chilling for from fifteen to twenty-four hours

A precaution absolutely necessary to obtain a satisfactory wax is the avoidance of high temperatures in evaporating the extract. Waxes prepared at temperatures above 60° C have yielded antigen emulsions giving false as well as true positive results. The antigen wax when properly prepared is a soft brown wax that keeps well in the refrigerator and at room temperature. With it, whenever desired, the antigen is made as detailed above

In addition to preparing the acetone insoluble antigen wax from a plain alcoholic extract of heart muscle powder, it has been obtained from an alcoholic extract after preliminary ether extraction (Kahn base) by concentration, acetone precipitation, and alcohol treatment as outlined above. The Kahn base itself, evaporated at 45° C, results in a soft brown wax lighter in color and more greasy than the acetone insoluble wax. When fanned at room temperature, the Kahn base is found to contain an oily substance in addition to a soft brown wax. It is possible that this oily substance is responsible for the nonspecific granularity that develops in the majority of Kahn antigen dilutions at low temperatures.

A plain alcoholic extract evaporated at 45° C results in a sticky brown wax which does not emulsify in salt solution. When fanned at room temperature, the plain alcoholic extract results in a sticky brown wax and an abundant transparent oily fat. When this residue is left at room temperature for a number of hours, a white precipitate settles out.

In contrast to the waxes just described that obtained by fanning (at room temperature) the alcoholic solution of the acetone insoluble antigen wax, contains no appreciable amount of oily substance but closely resembles the soft brown wax obtained at 45° C.

The solutions of these waxes likewise differ in character. Plain alcoholic antigens or those following preliminary acetone or ether extraction or those made by adding known substances to an alcoholic extract of previously extracted tissue, prepared a number of times according to a given formula, vary greatly in substances contained and in concentration of desired substances, not a few being unsatisfactory for use. The antigen prepared as described above, however, being of uniform quality and of known concentration, gives best results in serum tests for syphilis especially in the microscopic slide precipitation test. Whereas the majority of Kahn antigen dilutions are unsatisfactory at low room temperatures because a nonspecific granularity develops emulsions with the acetone insoluble antigen remain clear. In addition they are relatively stable and are therefore superior in use to the unstable Kahn antigen dilutions. For these reasons, the former technique of doing the microscopic slide precipitation test for syphilis in a warm room with ingredients and glassware warm and within a limited time to insure satisfactory results with the Kahn antigen dilution, has been simplified to the doing of the test with the acetone insoluble emulsions without any precautions relating to the temperature of the room glassware and ingredients or to the time (that day) the emulsions are prepared.

In working out the formulas described above for the precipitation test it was found that not only are the quantities of the ingredients important but also the order of their mixture. It is possible to secure uniformly satisfactory emulsions only when the cholesterol is precipitated before the antigen is added.

It was further observed that the sensitivity of the antigen emulsion is dependent more upon the cholesterol content than upon that of the antigen.

TABLE II
SERUM TESTS FOR SYPHILIS WITH CLINICAL COMPARISON

LABORATORY	SLIDE TEST VERY SENSITIVE EMULSION		SLIDE TEST SENSITIVE EMULSION		WASSERMANN TEST*		ANTICOM- PLEMENTARY
	Agreement	Dis- agreement	Agreement	Dis- agreement	Agreement	Dis- agreement	
Lakeside Hospital Courtesy of Dr A R Moritz	83	0	82	1	77	,	1
Mount Sinai Hospital	345	4	170	4	330	14	13
Total	428	4	251	8	412	19	14
	99.07%	0.93%	97%	3%	90.99%	4.41%	3.15%

*Cleveland method

Disagreements all in luetic sera ± to + + + + in one test negative in another

itself. Satisfactory emulsions may contain in a total of 44 cc, from 0.0375 to 0.15 cc of the antigen. The cholesterol content on the other hand, must be more carefully titrated since the more nearly it approaches the maximum dispersible in a given total, the more sensitive the emulsion becomes. Beyond this point the emulsions are unsatisfactory because of the presence of a nondispersible precipitate.

It was noted that distilled water has a greater dispersing effect on emulsions than has salt solution. On the other hand emulsions containing salt solution, although losing their antigenic properties sooner, give stronger precipitation reactions than those prepared with distilled water.

Concerning the influence of alcohol on the wax or in the emulsions, it was found that preparations from the concentrated alcoholic solution of the base (antigen) are most satisfactory since they permit of the dispersion, in a given total, of much more cholesterol than those prepared with the same quantity of the base in from five to ten times the quantity of alcohol. Furthermore they are relatively stable keeping (at room temperature) their antigenic properties undiminished for three days. Much more stable than these but less satisfactory for routine preparation are emulsions made from the wax itself (no alcoholic dilution). These (at room temperature) retain their antigenic power for weeks (see Table I).

The optimal quantities of the ingredients for the Wassermann antigen emulsions have not yet been determined. The emulsions described above however give good results. Titration shows the following:

	HEMOLYTIC UNIT	ANTICOMPLEMENTARY UNIT	ANTIGENIC UNIT
Very Sensitive Emulsion	Greater than fifteen times amount used in the test	Four to six times amount used in the test	One fourth amount used in the test

The results with the emulsions in the precipitation tests and Wassermann tests are given in Tables II to V.

TABLE III
COMPARISON OF MICROSCOPIC SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS

LABORATORY	SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (CLEVELAND METHOD)			SLIDE TEST SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (CLEVELAND METHOD)			SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (CLEVELAND METHOD)		
	Relative Agreement		Disagreement	Relative Agreement		Disagreement	Relative Agreement		Disagreement
	Agreement	Agreement	Disagreement	Agreement	Agreement	Disagreement	Agreement	Agreement	Disagreement
Lakeside Hospital	77	2	4	79	1	3	80	3	0
Courtesy of Dr A R Moritz	318	29	15	80	12	6	177	19	4
Mount Sinai Hospital	422	23	7						
City Health Department,	817	54	26	159	13	9	257	23	4
Courtesy of Dr H J Knapp	91 08%	6 02%	2 9%	87 85%	7 18%	4 97%	90 81%	7 78%	1 41%
Total	871	172	9	95 03%	9	4 97%	279	98 59%	4 41%
	97 1%		2 9%						1 41%

TABLE IV
SERUM TESTS FOR SYPHILIS WITH CLINICAL COMPARISON

DATE	SLIDE TEST		SLIDE TEST		SLIDE TEST		WASSERMANN		WASSERMANN		WASSERMANN		ANTI COMPLE MENTARY
	VERY SENSITIVE ANTIGEN EMULSION		KAHN ANTIGEN DILUTION		SENSITIVE ANTIGEN EMULSION		ETH IN SOL NONCHOL		ACETONE IN SOL CHOL		ACETONE IN SOL NONCHOL		
	Agree ment	Disagree ment	Agree ment	Disagree ment	Agree ment	Disagree ment	Agree ment	Disagree ment	Agree ment	Disagree ment	Agree ment	Disagree ment	
4/29/27 to 5/13/27	225	4	225	4	175	7	137	6	195	11	161	12	13
7/22/27 to 8/2/27	120	0			175	7			195	11	161	12	13
8/5/27 to 8/16/27	345	4	225	4	175	7	137	6	195	11	161	12	13
TOTAL	98 85%	1 15%	98 25%	1 75%	96 15%	3 85%	95 99%	4 01%	94 66%	5 34%	93 06%	6 94%	
Disagreements All in luetic sera ± to + + + + + in one test negative in another													

Disagreements All in luetic sera ± to + + + + in one test negative in another

TABLE V
COMPARISON OF SERUM TESTS FOR SYPHILIS

DATE	SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (ETH IN SOL. NONCHOL. ANTIGEN)		SLIDE TEST KAHN ANTIGEN DILUTION AND WASSERMANN TEST (ETH IN SOL. NONCHOL. ANTIGEN)		SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (ACET IN SOL. CHOL.)		SLIDE TEST SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (ACET IN SOL. NON CHOL.)		SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND KAHN ANTIGEN DILUTION		SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND SLIDE TEST KAHN ANTIGEN DILUTION	
	Agree ment	Relative agree ment	Agree ment	Relative agree ment	Agree ment	Relative agree ment	Agree ment	Relative agree ment	Agree ment	Relative agree ment	Agree ment	Relative agree ment
4/9/27 to 5/13/27	100	22	100	12	100	21	80	12	210	19	177	19
7/22/27 to 8/2/27		11		12		38%		6		2		4
8/1/27 to 8/16/27	108	7	100	12	7	7	80	12	210	19	177	19
TOTAL	318	9	318	38%	318	38%	81	12	90	13%	89	13%
	97.84%	8.01%	97.84%	9.42%	97.84%	9.42%	81.03%	12.25%	90.91%	8.22%	89.50%	9.5%
	14	1	21	12	184	7	92	6	29	2	196	4
	9.13%	4.1%	94.03%	3.8%	96.34%	3.66%	9.88%	6.12%	99.13%	97%	99%	2%

SUMMARY

1 Antigen emulsions, quantitatively prepared from an acetone-insoluble lipid base, obtained within a few days as described above, give better results in the microscopic slide precipitation test for syphilis than Kahn antigen dilutions and give good results in the Wassermann test

2 Formulas giving the optimal quantities of the ingredients for the precipitating emulsions are detailed above. The order of mixture in preparing these emulsions is likewise important. It is possible to secure uniformly satisfactory emulsions only when the cholesterol is precipitated before the antigen is added.

3 The emulsions for the microscopic slide precipitation test prepared as described above have replaced Kahn antigen dilutions in this laboratory for the following reasons:

Antigen Emulsions

(a) Relatively stable, satisfactory for at least three days

(b) May be used immediately after preparation

(c) Satisfactory at low room temperatures as well as at ordinary ones

(d) The very sensitive emulsion is more sensitive than the Kahn antigen dilution and no less specific

(e) The emulsions are more uniform in quality and quantity than the Kahn antigen dilutions

Kahn Antigen Dilutions

(a) Unstable, satisfactory for an hour or an hour and a half at most, usually less

(b) Must stand from ten to fifteen minutes before use

(c) Majority unsatisfactory at low room temperatures

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

TABES DORSALIS Atypical Tabes Dorsalis (Forme Fruste) Bennett, A. E. *Am Jour Med Sc* October 1925 cxcv, 538

Atypical or "forme fruste" type of tabes is the most frequently mis-cd form of the disease. Gastric crises in this variety often lead to unnecessary abdominal operations.

Incomplete histories, careless examinations and infrequent spinal punctures in doubtful cases account for most errors. Gastric crises occur in from 10 to 20 per cent of all cases of tabes dorsalis.

Reliable reports indicate that more than 10 per cent of the tabetics have needless operations performed at least once upon a mistaken diagnosis. There are frequent reports in the literature of patients having had four or five abdominal operations when the cause of the symptoms was nerve root pains of syphilis.

The classical signs of tabes are absent in approximately one-half of the early cases when abdominal pains may be present. The knee jerks are normal or exaggerated in at least 25 per cent of early cases. The same applies to the Argyll Robertson phenomenon and other later signs. Approximately 10 per cent of cases remain atypical throughout the entire course of the disease.

A complete life story of the individual with a careful analysis of the onset and development, a careful neurologic examination and a spinal fluid study should be made where there is the slightest doubt as to a diagnosis.

In patients presenting recurrent abdominal attacks with no relief from previous operations, the possibility of an atypical form of tabes dorsalis should be very firmly impressed upon the minds of surgeons in order to decrease the all too frequent incidence of unnecessary operations upon tabetics.

TOXEMIAS OF PREGNANCY Lactic Acid in the Toxemias of Pregnancy Stander, H. J. and Radelet, A. H. *Bull Johns Hopkins Hosp*, August 1926 xxxix No 2, p 91

In pregnancy complicated with low reserve kidney the lactic acid in the blood is increased. This accumulation of lactic acid disappears as the patient improves.

In pregnancy complicated with nephritis there is a similar accumulation of lactic acid in the blood which disappears with the improvement of the patient.

The factors which may possibly play a role in the accumulation of lactic acid in the two types of pregnancy toxemia are:

- Decreased elimination of lactic acid
- Decreased oxidation and interference with the resynthesis of lactic acid into glycogen
- A possible disturbance of the hydrogen ion concentration of the blood

HODGKIN'S DISEASE Unique Features of Hodgkin's Disease (Lymphogranulosis) Barron, M. *Arch Path and Lab Med* November 1926 ii No 5 p 659

From a study of 27 cases (including 23 autopsies) the following conclusions are reached: Hodgkin's disease is twice as common among males as among females. Fifty per cent of the cases occur during the second and third decades of life, although cases can occur in practically all ages. The average duration of this disease is under two years.

An associated infection with tuberculosis is not infrequent. The retroperitoneal and prevertebral lymph nodes are frequently involved, in our series exceeding that of the cervical nodes. The involvement of the spleen is a characteristic feature of this disease, and, contrary to the general opinion, this enlargement may at times be enormous. Involvement of the vertebrae may lead to a diagnosis of Pott's disease. No tissue or organ of the body is exempt from involvement.

The characteristic histologic picture is identical for every organ and tissue of the body. The Dorothy Reed cells or Sternberg's giant cells are characteristic of the disease.

The blood picture is not characteristic. Some cases, however, present marked eosinophilic leucocytoses, and this when associated with an enlarged spleen or enlarged lymph nodes is helpful in suggesting a correct diagnosis. A severe anemia of the secondary type is present in practically all cases in the terminal stages of the disease.

Some cases of Hodgkin's disease present characteristic types of chronic relapsing fever which probably constitute an integral part of the syndrome and are not due to secondary infections. A case of Hodgkin's disease with Pel-Ebstein type of fever is here reported which is unique in the regularity of the recurrences of the febrile attacks.

Pruritus is a common cutaneous manifestation in Hodgkin's disease, occurring in from 10 to 20 per cent of the cases. It may appear early, at times as an initial symptom, and may precede by months or even years the appearance of any visible skin lesions, when such lesions occur.

Hodgkin's disease (lymphogranulomatosis) is an entity anatomically as well as clinically. It is an infectious granuloma caused by an organism not yet identified.

Reasons are here presented to suggest that the etiologic agent is probably an animal parasite.

Neither tuberculosis nor the tubercle bacillus bears any etiologic relationship to this disease.

Caseation necrosis may be extensive in the lymph nodes of Hodgkin's disease and may thus resemble the necrosis of tuberculosis and lead to a mistaken diagnosis.

The pseudodiphtheria bacillus—*B. hodgkini*—has no casual relationship to this disease.

The prognosis of this disease is always hopeless. No chronic infectious disease caused by any known bacterium is so invariably fatal.

Röntgen ray therapy may cause temporary symptomatic relief, but apparently has no effect on the progress of the disease.

An incontrovertible diagnosis can be established only through a biopsy.

NEUROSYPHILIS AND MALARIA Treatment of Neurosyphilis by Malaria, O'Leary, P. A., Goeckerman, W. H. and Parker, S. T. Arch. Dermat. and Syph., November, 1926, 117, No. 5, p. 550.

A report of the second year's observations on thirty-five patients treated by malarial infection and on an additional sixty-five patients treated during the past year.

The continued observations on the thirty-five patients, treated by the fever therapy of Wagner von Jauregg and followed for a period of two years, has convinced us of the value of the method in the treatment of parenchymatous neurosyphilis, particularly of the general paralytic type. It also suggests that some of the complications of neurosyphilis, such as gastric crisis, persistent lightning pains and optic atrophy, will be ameliorated by fever therapy. There has been a mortality of 8 per cent in the 100 patients treated, of which five deaths (5 per cent) may be attributed, directly or indirectly, to the malaria.

Although there has been a slight increase in the number of remissions produced during the second as compared with the first year, the outstanding feature of the study has been that thus far none of the patients who were improved has shown any evidence of relapse, but all have continued to improve. Also, there has been a reduction in the degree of positiveness of the serologic tests of the blood and spinal fluid in the group as a whole. The outstanding serologic observation has been the absence of serologic relapse in any of the patients reexamined. The authors' experience has taught them to favor the use of the present day antisyphilitic remedies three or four months after the course of malarial fever.

on the observation that those patients who received intrapinal treatment for example after the course of fever, have shown better clinical and serologic results than those who did not

They believe that some of the therapists who have been disappointed in the fever therapy of Wagner von Jauregg are discouraged because the signs and symptoms of the advanced phases of the disease and the result of actual destruction of tissue have not been obliterated. The authors have not anticipated such phenomena but have been encouraged by obtaining definite symptomatic benefit. In their previous communication it was pointed out that the patients with general paralysis were in the early phases of the disease and not far enough advanced to warrant institutional care.

The authors are now more enthusiastic about the results of the treatment with malaria than they were last year. Their past experience however with the treatment of neurosyphilis, particularly early general paralysis, prevents them from making conclusive deductions at this time of the value of the method. A more extended trial with frequent observations is warranted by the results thus far obtained.

WASSERMANN REACTION The Occurrence of the Wassermann Reaction After Milk Injections Kirschenblatt D and Narinjan A. Deutsch med Wchnschr July, 1926, 11, 1298

This investigation was stimulated by the article of Felix Klopstock in the Deutsch med Wchnschr 1925 No 41. In a number of patients with different acute and chronic affections after excluding syphilitics and strict control by the Wassermann reaction an attempt was made to find a reaction corresponding to the syphilitic blood change by means of several milk injections. Fifteen patients among them four women were given preliminary treatment with boiled cow's milk every three to four days in doses of 2, 5, 8 and 10 cc.

With two exceptions all the patients showed general reactions and fever often high (up to 39 C), in the arthritics and rheumatics there were also focal reactions. And yet there was not a Wassermann reaction in a single case the blood being examined both in the febrile stage and in the resting interval between the injections eighteen to thirty hours after each milk injection. The Wassermann reaction was performed with two antigens, a specific one and a normal one (orchest by Bordet Rouleux method). These attempts to bring about a positive Wassermann reaction even temporarily in diseased individuals by the parenteral injection of milk were negative.

SICKLE CELL ANEMIA The Sick Cell Phenomenon Cooley T B and Lee P. Am Jour Dis Child, September, 1926 XXXII 334

Of 400 colored patients (children) 30 or 75 per cent were found to have sickle cells. Two new facts are added. (1) In preparations kept at incubator temperature the sickle cells disappear rapidly, leaving the round cells behind. (2) The cells of one of the patients having a hemolytic anemia are rapidly hemolyzed at incubator temperature in their own serums and in serums from normal bloods while the serum of the patient was not hemolytic for normal cells.

It is suggested that these findings imply a special vulnerability of the sickle cell.

The authors consider the sickle cell to be a congenital familial and hereditary phenomenon possibly transmitted chiefly through the mother.

They believe also, that the presence of sickle cells in the blood does not in itself imply any essential anemia active or latent. Most of the patients with sickle cells are as normal in all other ways as the rest of the colored population in Detroit. The term 'sickle cell anemia' should be reserved for patients with definite hemolytic anemia and another term used for the condition without symptoms. 'Sicklemia' is suggested.

They believe that sickle cell anemia is a true disease entity to be considered as another form of familial hemolytic jaundice and that there may be a hemolytic 'diathesis due probably to a special vulnerability of the sickle cells to some hemolytic agent.

They speculate as to the possible sequelae dependent upon a consideration of 'sicklemia' as a dominant mendelian characteristic.

CATARRHAL JAUNDICE The Pathology of Icterus Catarrhalis, Klemperer, P, Kilian, J A, and Heyd, C G Arch Path and Lab Med, November, 1926, 11, No 5, p 631

The so called "icterus catarrhalis" is neither a morbid nor a pathologic entity. Three forms can be differentiated

1 Icterus due to obstruction of the common duct following gastrointestinal catarrh—true catarrhal jaundice

2 Icterus due to degeneration and multiple necrosis of the liver, hematogenous in origin

3 Icterus due to cholangitis, mostly of hematogenous origin

The etiologic factor of group 2 is not known, bacterial toxins of various types have to be considered. It is probable that in group 3 atypical strains of *B. paratyphosus* are of etiologic importance. It is possible that groups 2 and 3 frequently merge with each other.

The evidence of hepatic derangement in cases of group 2 suggests careful observation of these cases and dietary regulations in order to prevent further damage to the liver. The history, the presence of urobilin in the urine and the positive results of practically all the liver function tests permit a differentiation from group 1. Further studies are necessary, however, in order to make a correct differentiation of groups 2 and 3 possible.

AUTOHEMOTHERAPY Effect of Autohemotherapy on the Blood Picture, Hirsch, L Deutsch med Wchnschr, July, 1926, 11, 1302

The author made a systematic study of autohemotherapy on the blood picture, giving 5 cc of the patient's own blood intramuscularly or subcutaneously or the same amount of the serum intramuscularly. There were cases of angina, grippe, bronchitis, asthma, pneumonia, pleurisy, chronic and acute polyarthritis. Blood smears were made and blood taken for leucocyte counts shortly before the injection and five, ten and fifteen minutes and three, six and nine hours afterward. No change in the blood picture due to digestion was seen in the smears taken two hours after meals. More importance was attached to the relative than to the absolute leucocyte counts, for often there is no parallelism between the absolute and relative counts.

In all 31 tests were made. In 12 of these there was no effect on the blood picture, 6 of these being after intramuscular injection, 5 after subcutaneous and 1 after injection of serum. Ten of these 12 were in the subjects with normal blood pictures, it is natural that bone marrow that is not affected by the disease should not react to the patient's own blood. Yet a minority of the patients with normal blood reacted to the injections. Blood pictures shifted to the left were affected in over 80 per cent of the cases, and in the majority both the lymphatic and myeloid part was affected. But in general there was less reaction of the lymphocytes than of the neutrophils. Among the 19 cases that reacted 13 showed an improvement in the blood picture (increase in lymphocytes, decrease in rod nucleated forms), in 6 it grew worse (increase of rod nucleated forms and decrease of lymphocytes). In the improved blood pictures there was generally an increase in the eosinophiles up to 8 per cent of the total leucocytes. No rule could be determined in the variations in the monocytes. Only changes of at least 25 per cent as compared with the beginning values were considered. Frequently the changes, particularly in the rod nucleated forms were 50 per cent or more.

The cause of the effect on the blood picture is probably a stimulant action of the injected blood or products of its catabolism on the bone marrow or on the distribution of the blood in the different organs. If it is a question of change only in the absolute counts it can be explained by changes in distribution. But where the proportion of the different kinds of cells is changed the bone marrow must be involved. The stimulus on the function of the bone marrow is probably brought about through the intermediation of the vegetative nervous system.

In the patients in whom the blood picture did not change or grew worse the clinical picture did not change noticeably in either direction. But even in the patients with an improved blood picture the clinical picture was improved in only 50 per cent. But the experiments were at first made with doses that are far less than those used therapeutically.

LABORATORY TECHNIC

FEVER AND SYPHILIS *The Therapeutic Effect of Fever in Experimental Rabbit Syphilis* Schamberg J F and Rule A M Arch Dermat and Syph September, 1926, *iv*, No 3, p 243

It is possible to prevent syphilitic infection in rabbits after testicular implantation of the specific virus if the rabbits are given a series of baths at a temperature of 45° C (113° F) within three to four days after inoculation. An average rise of temperature of 4° F is induced.

How many baths are necessary to sterilize the infection is not yet definitely known, but at the date of this communication it would appear that about nine are necessary.

Infection can also be prevented by heating the spirochetic suspension on a water bath at 40° C (104° F) for one hour before inoculation.

It would appear that the thermal death point of the body of *Spirocheta pallida* is about 41° C (105.8° F) with an exposure of approximately six hours.

While these experiments indicate that *Spirocheta pallida* in the rabbit cannot withstand high fever, it is not definitely proved that this applies to human syphilis.

Whether these experiments shed any illumination on the mode of action of malaria in syphilis can only be determined by further investigation.

CARCINOMA *Grading the Malignancy of Carcinoma and Its Practical Application* Broders A. C Arch Path and Lab Med September 1926 *ii* 3, C

Broders uses the following classification:

If an epithelioma shows a marked tendency to differentiate, that is, if about three-fourths of its structure is differentiated epithelium and one-fourth undifferentiated, it is graded 1. If the differentiated and undifferentiated epithelium are about equal, it is graded 2. If the undifferentiated epithelium forms about three-fourths and the differentiated about one-fourth of the growth, it is graded 3. If there is no tendency of the cells to differentiate, it is graded 4. Of course the number of mitotic figures and the number of cells with single large deeply staining nuclei (one-eyed cells) play an important part in the grading.

He further modified this conception as follows:

Instead of a Grade 1 epithelioma in which about three-fourths of the cells are differentiated and one-fourth undifferentiated, should be substituted a Grade 1 epithelioma one in which differentiation or self control ranges from almost 100 to 75 per cent and undifferentiation from almost nothing to 25 per cent. A Grade 2 epithelioma one in which differentiation or self control ranges from 75 to 50 per cent and undifferentiation from 25 to 50 per cent. A Grade 3 epithelioma one in which differentiation or self control ranges from 50 to 25 per cent and undifferentiation from 50 to 25 per cent. And a Grade 4 epithelioma, one in which differentiation or self control ranges from 25 per cent to practically nothing and undifferentiation from 75 to practically 100 per cent. So far as estimation of the ultimate result is concerned, this revision will have no effect on Grades 1 and 2, but will affect slightly Grades 3 and 4 because a small percentage of neoplasms formerly classified Grade 3 will now be classified Grade 4. In other words, the most malignant of the Grade 3 neoplasms will be classified in Grade 4.

He calls attention to the practical application of this procedure in these words:

'Turning to the practical side of the grading of cancer, it is well known that cancer of Grade 1 shows practically no tendency to metastasize and therefore in dealing with such neoplasms it does not seem necessary to remove the regional lymph nodes. This saves the patient unnecessary operative procedures. As practically all cancers of Grade 4 with metastasis prove fatal sooner or later, the patients should not be subjected to an operation involving the regional lymph nodes unless they are in close proximity to the primary growth, cancer of the stomach for example. Judd New and Figs believe it is useless to perform block dissection of the neck in the presence of a Grade 4 epithelioma of the

lip, tongue, cheeks, floor of the mouth or nostrum, etc. In cases of cancer of Grade 2 and in a certain proportion of those of Grade 3, with metastasis, removal of the regional lymph nodes offers a permanent cure in a fair number of cases, as evidenced by the fact that ten (33.3 per cent) of thirty patients with squamous cell epithelioma of the lip of Grades 2 and 3, with metastasis in one group of submaxillary lymph nodes, were living and well on an average of six and one fifth years after removal of the nodes."

TUBERCULOSIS AND ASPERGILLOSIS *Aspergillosis of the Lungs and Its Association with Tuberculosis*, Lapham, M E Jour Am Med Assn, September 25, 1926, LXXXIII, 1032

Lapham summarizes from the literature the reports of pulmonary aspergillosis in human beings, calls attention to the difficulty of differentiating this condition from tuberculosis and expresses the belief that it is a more frequent complication or concomitant of tuberculosis than is recognized. She argues for routine cultures of the sputum in tuberculosis and says:

"Here is a disease that is capable of causing pleurisy, acute and chronic, bronchitis, acute and chronic, pneumonias, acute and chronic, emphysema, bronchiectasis, atelectasis, sclerotic fibrosis, tubercles, cavities, endarteritis, thrombosis, infarcts, hemorrhages, asthma. Would it be strange if such a disease should seriously complicate or even inhibit recovery in a case of tuberculosis?"

She concludes that we are thoroughly ignorant of the frequency of aspergillosis both as a primary and as a secondary disease.

We have no idea how much aspergillosis of the lungs predisposes to tuberculosis in human beings or in cows.

We do not know how much it impedes or even inhibits recovery in cases of tuberculosis.

We have no idea whatever as to its association with acute respiratory diseases.

We know that it affects cows much as it does human beings.

We know that it gives the same reaction to tuberculin that tuberculosis does.

Should we apply this knowledge to the study of the tuberculosis of dairy herds?

In order to gain adequate information as to the frequency and importance of this disease, should not a systematic research study be made:

1 By determining the percentage of aspergillosis cases among the tuberculosis cases in the large tuberculosis sanatoriums.

2 By determining the percentage of cases of aspergillosis in the lungs at necropsies.

3 By determining the percentage of cases of aspergillosis in cases of respiratory diseases.

4 By applying these principles to dairy herds.

MEASLES TOXIN Its Preparation and Application as a Skin Test, as an Immunizing Agent, and for the Production of an Antitoxin, Ferry, N S and Fisher, L W Jour Am Med Assn, March 27, 1926, LXXXVI, 932

The organism investigated was isolated in pure culture from the blood of patients in the early stages of the disease. It is a Gram positive, aerobic, green producing streptococcus appearing both in pairs and in chains. It differs from the organism described by Tunnicliff in growing luxuriantly in the presence of oxygen and in producing a soluble toxin specific for measles.

Isolation was accomplished by planting 10 cc of venous blood in 0.25 liter flasks of Hibler medium and, after incubation for twenty four hours, plating directly on sheep blood agar. After twenty four hour incubation the plates were planted to blood agar slants or 0.2 per cent broth.

The organism has been named tentatively *Streptococcus morbilli*.

The Berkefeld W filtrate from a six day growth in 0.2 per cent glucose broth contained a soluble toxin.

Of 35 persons with no history of measles, 14 gave a positive skin reaction to the filtrate when injected intradermally in 0.1 cc.

Of 30 children with a positive measles history none reacted positively, two adults of 30 with positive histories reacted positively.

Positive reactions were obtained in the preeruptive and early stages of measles while negative reactions occurred in the later and convalescent stages.

When mixed with convalescent serum no reactions were obtained.

When mixed with artificially prepared antitoxin no reactions were obtained in susceptible individuals.

When mixed with scarlet fever antitoxin the measles toxin was not neutralized.

Persons giving positive reactions are made to give negative reactions by the injection of measles convalescent serum.

Serum from measles cases agglutinates this streptococcus.

Intracutaneous injection into rabbits produced a rash suggestive of measles.

Animals injected with measles toxin produce serum with antitoxic properties.

CANCER SERUM The Reducing Power of Cancer Serum Mondain C Dauris R and Beck J Compt rend Soc de biol April 23 1926 vol 963

Thomas and Binetti having suggested that cancer serum might be distinguished from normal serum by the rapidity with which it reduced methylene blue when mixed with an extract of cancer tissue, the authors studied the mechanism of this reaction and conclude that fresh cancer serum in the absence of neoplastic extract is incapable of decolorizing methylene blue. Thomas and Binetti's extract decolorized methylene blue by itself. The mechanism of the reaction proposed by Thomas and Binetti for the serum diagnosis of cancer is not therefore due to a high degree of reducing power in the cancer serum. It is due simply to the reducing action of bacteria and their secretions the bacteria necessary to the production of the reaction being found in the extract which is used for the reaction.

MENINGITIS A Case of Sporotrichosis Meningitis Hyslop G H Neal J B Kraus W M and Hillman, O Am Jour Med Sc November 1926 vol 11 No 5 p 726

Sporotrichosis infection of the nervous system has not been observed previously.

The patient was a girl fifteen years old who became ill December 30 1924 and died June 19 1925. The infection involved the posterior fo so largely had a febrile onset, and ran a chronic remitting course. There were slight exacerbation about every ten days accompanied by a slight fever.

Interesting clinical occurrences were tetanoid attacks, micropsic hallucinations and several temporosphenoidal seizures.

The diagnosis was made by the presence of sporotrichia spores and mycelial forms in the spinal fluid.

Iodid therapy was of no avail. Experiments with radium intravenously gave interesting results.

Culture and animal inoculation results were negative.

Microphotographs of the spores seen in the spinal fluid and of the tissue from the patient's brain are exhibited.

Autopsy of the patient showed a chronic diffuse leptomeningitis most marked in the posterior fossa, a moderate edema of the brain and the presence of a peculiar gelatinous exudate here and there beneath the pia. Microscopically there was shown a chronic productive leptomeningitis but no inflammatory involvement of the brain substance.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*Outlines of Common Skin Diseases**

THIS little book is intended to serve as a simple and concise guide to the diagnosis of the more common diseases of the skin. It is in no sense of the word a textbook, nor is it intended to be, but rather an outline suggesting a systematic course of procedure from which the student or practitioner may go to larger texts for detailed information.

A short classification, embracing the common skin diseases only, is followed by a very practical outline of the method of making an examination and thus by a table of regional distribution. The common skin diseases are then outlined, classed in accordance with their primary lesions.

The little book contains a large amount of information and is, in effect, a set of student's notes.

Scarlatina is listed as among the diseases of unknown etiology in spite of the rather general acceptance of the work of the Dicks.

There are a few typographic errors, such as "a womep" for "a woman" on page 48.

In the endeavor to be concise the style has become somewhat jerky and almost too condensed, and occasional abbreviations, such as "mercurial stomat" seem unnecessary.

It is unusual to see so small a book so profusely illustrated.

Basal Metabolism in Health and Disease†

WHILE the subject of basal metabolism has become of considerable importance to the practitioner of medicine within recent years, like all subjects which are in the course of development, much of the literature has been written primarily for research workers and physiologists. In this book Dr DuBois, whose relation to the development of basal metabolic studies needs no comment, has brought basal metabolism into the domain of clinical medicine and has presented, for the student and the practitioner, a comprehensive, clearly written, and eminently practical review of this important subject.

The volume, a second edition, has been thoroughly revised throughout.

Beginning with a brief but thorough historical review, the metabolism of carbohydrates, fats, and proteins is next discussed in an interesting and understandable manner, the physical laws related to the calculation and understanding of metabolic rates are next covered, after which the gases of the body and their relation to internal and external respiration are thoroughly discussed.

Following is an explanation and review of the general principles of respiration apparatus, and the methods of calculation involved in their use concerning which Dr DuBois emphasizes that "the man who conducts metabolism experiments should be perfectly frank with himself regarding the accuracy of his work. He should estimate the possible percentage of error and never try to conceal from himself the fact that the technic of a certain experiment was rather poor."

**Outlines of Common Skin Diseases* By T. C. Gilchrist, Clinical Professor of Dermatology, Johns Hopkins University. Cloth. Pp. 54 with 53 illustrations. Williams & Wilkins Co. Baltimore.

†*Basal Metabolism in Health and Disease* By E. F. DuBois, M.D., Associate Professor of Medicine, Cornell Second Edition. Cloth. Pp. 431 with 92 illustrations. Lea and Febiger Philadelphia. 1927.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

After a review of the methods and underlying the estimation of the body surface area the factors which may influence the normal basal metabolism are discussed in chapter VII, and the following chapter covers a consideration of the selection of normal standards. These chapters are extremely valuable as clarifying the interpretation of basal metabolic readings and should be read by every practitioner interested in the subject or attempting to utilize the application of this method of examination to the study of disease.

After a brief review of the theories of basal metabolism the remainder of the text (Part II—208 pages) is devoted to the significance of basal metabolic determinations in disease.

The relation of undernutrition, overnutrition and obesity to basal metabolism is reviewed in an interesting and practical manner following which 46 pages are devoted to the basal metabolism in diabetes and 56 pages to its relation to thyroid diseases.

The remainder of the book is given to a discussion of basal metabolism and the adrenal, pituitary, and sex glands, basal metabolism in diseases of the blood, in diseases of the heart and kidneys and in fever, water metabolism and the influences of diseases of the nervous system on the basal metabolism.

This is a book which few can afford to be without.

The Chemical Aspects of Immunity

A COMPREHENSIVE monograph by the author of *Chemical Pathology* of exceeding interest to chemists and immunologists.

Manual of Operative Surgery†

THE sixth edition, thoroughly revised, of a well known handbook of operative surgery clearly written, excellently illustrated, and comprehensive in scope though compact in form.

The Heart‡

IN THIS little volume the author has endeavored to summarize in a brief but clear fashion the main principles of cardiology.

It is intended for students and for practitioners who desire to review rapidly the modern views of the cardiovascular system.

Its chief value should be as an impetus leading to more extended and comprehensive studies of this important and complicated subject.

Bacteriology for Nurses§

ANYONE who has attempted to teach bacteriology to nurses will appreciate the difficulties involved, especially in view of the short time allowed by the curriculum for the presentation of this and allied subjects. It is very debatable whether or not many subjects now included in the nursing curriculum and especially the extent to which they are supposed to be covered, are really necessary for the training of a nurse and it is relatively certain that a satisfactory working knowledge of them is more often the exception than the rule.

Certainly, however, a knowledge of the relation of bacteria to the production of disease and some conception of the mechanism involved in the prevention of disease is essential.

The Chemical Aspects of Immunity By H. Gideon Well, Professor of Pathology, University of Chicago. Cloth. Pp. 241. The Chemical Catalog Co. New York 1925.

†*Manual of Operative Surgery* By Sir H. J. Waring, Examiner in Surgery, University of Oxford. Cloth. Pp. 864 with 618 illustrations. Price \$5. Oxford University Press, 1927.

‡*The Heart* By A. G. Gibson, Physician to the Radcliffe Infirmary. Cloth. Pp. 106 with 14 illustrations. Oxford University Press, 1926.

§*Bacteriology for Nurses* By H. Fox, M.D., Director, William Pepper Laboratory, University of Pennsylvania. Fourth edition. Cloth. Pp. 212 with 67 engravings and 7 colored plates. Lea and Febiger, Philadelphia 1926.

for the intelligent care of the sick To supply such information is the purpose of Dr Fox's little book

Textbooks of bacteriology for the use of nurses are likely to fall into two errors they are either too comprehensive and technical to come within the field or the comprehension of the nurse, or so elementary as to remind one of the bed time story

Dr Fox has endeavored, and with some success, to find a middle road The volume is attractively printed and very well illustrated and has been revised to include the more recent advances in this subject

Principles of Physical Chemistry[†]

AS IS stated in the introduction to this book "Even the most exalted members of the human family play their parts against a background of semipermeable membranes separating watery solutions of salts, proteins, and other substances," so that, "it is necessary, therefore, that those who some day may be called upon to diagnose and to heal their ills should know something of the complex of chemical events which make up life"

The usual textbook of physical chemistry is too large and too abstract for the needs of the medical student, and the author has endeavored, therefore, to compress into a small volume, a strictly utilitarian text which covers the essentials necessary for an understanding of a complex subject applicable to its relation to the study and practice of medicine

The book is divided into two main parts (1) theoretical and (2) practical, embodying a series of laboratory exercises

The volume is quite practical, well and clearly written, and should be of great use to the student and as a source of reference and review to the practicing physician

Allergic Diseases[†]

IN THIS small volume the author records the results of his investigations of allergic diseases as encountered by him in Holland since 1919

In view of the fact that he found sensitization to Walker's allergens to be relatively infrequent, he developed certain "non specific" methods of treatment which are given in detail and from the use of which 50 per cent of cures were obtained in asthma, 30 per cent of cases being improved, and 20 per cent uninfluenced, somewhat better results being obtained in hay fever and migraine

As a result of his studies in Holland and Switzerland Van Leeuwen is convinced that in other low regions as well, influences of climate are the main causes of asthmatic and allergic attacks, these climatic influences being identified with a particular sort of allergens (climate allergens or miasms) occurring in the air in lower countries and absent or present only in minor degree in higher altitudes

These climate allergens are, for the most part, decomposition products of various materials, or products of the metabolism of various animal or vegetable parasites, the occurrence of which is due to the influence of climate, temperature, humidity of the air, etc

The book is, in essence, the story of the investigations upon which these etiologic concepts are based and a recital of the various methods of treatment which the author has developed, among them the use of tuberculin and, particularly, the isolation of patients in a "miasm free" chamber

The author believes that an important factor in the etiology of allergic conditions is an increased permeability or vulnerability of the skin and mucus membranes and that allergic diseases are caused by the inhalation or ingestion of toxic substances through such vulnerable spots, the two important factors being increased permeability and deficient immunization

The final seventy-two pages of the book are devoted to a detailed account of the author's methods of treatment, including methods directed toward the production of anti

*Principles of Physical Chemistry for Medical Students By P M T Kerridge Cloth Pp 134 with 22 illustrations Oxford University Press 1927

†Allergic Diseases By W Storm Van Leeuwen M D Director Pharmacotherapeutic Institute Leyden Holland Cloth Pp 142 with 3 illustrations J B Lippincott Co Philadelphia

anaphylaxis, skeptophylaxis, specific and nonspecific methods including the use of tuberculin, milk, sulphur peptone etc., and the author's 'miasm free chamber'

The book is of interest to all engaged or interested in the study of allergic diseases and will undoubtedly be read

Introduction to Physiologic Chemistry[†]

IN THIS volume the author has endeavored to present in a coherent manner the main aspects of physiologic chemistry as correlated with recent advances in this subject

Laboratory methods and tests are omitted as the purpose of the book is to present a relatively brief, but yet sufficiently comprehensive review of the subject to serve as a source of fundamental principles

The text is clearly written and evidences an assiduous search of the pertinent literature As a source of reference the book should prove useful to the student and practitioner, to whom it is addressed

Elements of Hygiene and Public Health[‡]

A SECOND edition of a well known English text which has been thoroughly revised Written particularly to fill the needs of the practitioner at large, the volume well fulfills the purpose for which it is intended

Manual of Medicine[‡]

THIS is the third edition extensively and thoroughly revised of a useful and comprehensive handbook of medical practice

Though brief in style the numerous conditions covered are well described in a clear and practical manner and the volume should be a useful desk companion

The Harvey Lectures (1925-1926)[§]

IN THIS volume are collected seven lectures delivered by different scientists before the Harvey Society in the years 1925 and 1926

The first (8 pages) by Professor E P Nager of the University of Zurich discusses various otologic problems and the theoretical and practical results of otologistologic laboratory work In the second lecture (40 pages) by Dr J N Northrop is presented a summary of investigations concerned with the dynamics of pepsin and trypsin the details of the work having been presented previously in the *Journal of General Physiology* (I to VI)

The third lecture (37 pages) by Dr W H Lewis discusses the transformation of mononuclear blood cells into macrophages epithelial cells and giant cells and is followed by a comprehensive survey (59 pages) of recent investigations concerned with the parathyroid glands by Dr J B Collins

Empiricism and rationalism a discussion of the proper method of treating data from the statistical and mathematical standpoint is next presented (4 pages) and followed by a lecture (15 pages) upon the historic outline of medical therapy delivered by Professor Knud Faber of Copenhagen

The final lecture (14 pages) delivered by Professor Brouwer of Amsterdam, surveys comparative anatomy and neuropathology

The volume is without an index but the typography is excellent

[†]Introduction to Physiologic Chemistry By M Balan ky Associate Professor of Physiologic Chemistry University of Texas Cloth Pp 440 with 40 illustrations John Wiley & Sons New York 1927

[‡]Elements of Hygiene and Public Health By Chas Porter Lecturer in Public Health Middlesex Hospital Medical School Cloth Pp 400 with 98 illustrations Oxford University Press

[‡]Manual of Medicine By A S Wock York Dean of the Medical School Westminster Hospital Third edition Pp 33 Cloth Oxford University Press

[§]The Harvey Lectures (1925-1926) Series XXI Delivered under the auspices of the Harvey Society of New York Pp 290 Illustrated Cloth Williams and Wilkins Co Baltimore

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EDITORIALS

The Nature of Allergens

THE developmental work in anaphylaxis and in the present status on the basis of the assumption that nature Antigens are related as those substances which are elaboration of a specific function it is to neutralize and destroy the said substance up to the present all antigens are protein protein appears to be the elaboration of a specific specificity of antigens dependent upon tion, and is determined of aromatic radicals the protein molecule acids are not complexes of d not antigen the aromatic tyrosine, be at least one formaldehyde and into the whole, the entire molecule

has grown to its are protein in when introduced body whose eight of overmori body

The purest known proteins act as antigens. Indeed, the purer the protein the more thoroughly it sensitizes the animal and the smaller is the dose necessary. Even crystallized proteins, such as hemoglobin, egg albumen, and edestin are antigenic.

Early efforts were to determine whether anything less than the whole protein molecule would serve as an antigen and if so, at what stage of its racemization the antigenic power will disappear. Vaughan and his coworkers have maintained that the protein molecule is made up of two factions, a sensitizing, nonpoisonous moiety, and a nonsensitizing, poisonous portion. The sensitizing faction is different in its chemical composition in every protein, while the poisonous faction is the same or nearly the same in all proteins. The nontoxic, sensitizing group appears not to be a biuret body. Zunz, likewise, believes that the amino acid groups responsible for sensitization are not the same as those producing shock. Gay and Robertson concluded that the property of specificity is exercised by certain chemical groups in the protein molecule. Wells and Osborne concluded that specificity is determined by chemical structure rather than by the biologic origin of the protein.

Even the earliest digestion of proteins lessens the antigenic activity. Until recently proteoses and peptones have been considered nonantigenic, but the probability is that proteoses are still antigenic. The tuberculin reaction is probably a proteose sensitization reaction. Gelatin, a hydrolytic protein, is nonantigenic, probably due to its lack of aromatic radicals. Obermyer and Pick have found evidence that the aromatic radicals may be of importance in determining the specific character of immunologic reactions. Wells states that the cleavage products of a protein even when all are injected together, have no antigenic capacity. When artificially reunited into colloidal molecules (plastons) their antigenic capacity may be restored. Wells states that in the hydrolytic cleavage of proteins the capacity to invoke antibody formation is lost somewhere between the intact state of the molecule and its complete separation into the constituent amino acids. But as yet we do not know just where. Although there have been many contradictory findings reported as to the antigenic capacity of proteoses, peptones and peptids, most of the statements have been made with little comprehension of the changes involved in protein hydrolysis or of the nature of the mixtures investigated. The so-called peptones are notoriously variable preparations. To make matters worse, much of the work has been controlled with the anaphylaxis reaction. Here the criteria of reaction are furnished by the behavior of an injected animal, and as many of the anaphylactic symptoms may be essentially reproduced by intoxication with protein cleavage products independent of any immunologic reaction, the value is dubious. A critical investigation and review by Fink emphasizes the worthlessness of much of the published evidence. There is no reason at present to assume that the so-called proteoses or peptones of various designations represent anything but the crudest mixtures, or to expect that constant results will be obtained in any investigation as to their properties.

Abderhalden claims to have produced sensitization with a synthetic polypeptid containing fourteen molecules of leucin and glycine. Zunz claims sim-

ilar results with simpler polymers. These statements are, however, not easily accepted, for the reactions were obtained after intravenous injection into anesthetized rabbits and consisted of fall in blood pressure, increased rate of respiration, and expulsion of feces and urine. These reactions, slight as they were, were not constant. Many of the reactions were quite negative. The plastens, previously referred to, are, on the other hand, true synthesized proteins produced from proteoses through the reverse action of proteolytic enzymes.

Numerous attempts have been made to produce anaphylaxis to nonprotein substances. This has never been conclusively demonstrated in animals. Early claims of anaphylactic shock to nonprotein substances have usually been discredited and found due to anaphylactoid reactions, thus, lipoids and agar have produced symptoms very much resembling acute anaphylaxis but due to blood changes, one of the most prominent of which consists of thromboses in the pulmonary circulation. However, this work has been carried out chiefly on experimental animals, and it is well known that different animals show the greatest variation of susceptibility.

Pick has found an apparently nonprotein substance in young typhoid cultures which, on injection, produces precipitins but no antibodies. Parker has found a similar substance in pneumococci, influenza bacilli, and staphylococci. Heidelberger and Avery have isolated a polysaccharide from the pneumococcus which causes the production of precipitin. They have found three polysaccharides, at least one of which is entirely nonnitrogenous.

Recently the question has again been raised whether the antigen in some cases of allergy is protein in nature.

There can be no doubt that pollens contain protein substances. Haehl has obtained an albumin, a proteose, and a glutelin from the pollen of ragweed. These proteins are, however, very feebly antigenic. Ulrich and Koesler have reported successful sensitization of guinea pigs with pollens, but Cook, Coca, and others were unable to sensitize guinea pigs.

They further tested 1 per cent and 5 per cent concentrations of ragweed pollen extract. The stronger contained only three times as much nitrogen but was actually ten times as potent when checked by nasal testing. The conclusion was that the active substance of pollen is not protein, that standardization by nitrogen content and by complement fixation does not determine the content of the active substance, and that treatment with protein free extract should be effective and at the same time free from the possible objectionable features of injection of extraneous protein. They found that the components which act as antigen when pollen extracts are used in complement fixation tests are removed by protein digestion but that the atopic activity was undiminished.

These authors in 1925 treated sixty-two ragweed sensitive persons with protein free extracts. These extracts gave negative biuret, Adamkiewicz, zanthoproteic and reduced sulphur reactions for protein. Eighty-three and nine tenths per cent of the series were practically free from symptoms.

Black points out that standardization of pollen extracts by nitrogen content is not reliable for there may be some proteins present which are not antigenic. Complement fixation as applied to pollen extracts is only another method of determining the protein in the extract and while it may serve to distinguish some proteins from others, it is entirely valueless as a means of estimating the amount of active substance.

Grove and Coca suggested that the active substance resembled an enzyme. Black has shaken ragweed extract for four hours in a mechanical shaker and found its activity unchanged. It has been frozen solid for six days and lost none of its activity. Boiling for ten minutes and heating in the autoclave at ten pounds for ten minutes did not affect it. The evidence does not support the assumption that the active substance is an enzyme. The former authors had previously reached the same conclusion.

Black believes that anaphylactic reactions may be secured in guinea pigs by the use of extracts rich in protein and poor in active substance but that these reactions, like complement fixation, are reactions to the protein of the extract and have no relation to the atopy or allergen.

The observations of Grove and Coca and of Black are of the utmost interest in that they again raise the question as to whether substances which produce clinical allergy or anaphylaxis need necessarily be protein in nature. Naturally the work requires confirmation and study from various additional angles. The question arises whether the nitrogen free extracts obtained after tryptic digestion were tested against nonsensitive controls to make certain that the subsequent reactions were not nonspecific in character. Presumably they are not, and presumably these control tests have been made.

We should bear in mind that Bernton and his co-workers have found protein derivatives in pollen which appear to be active in extremely minute amounts. One gram of timothy pollen yielded 0.0031 grams of proteose A, 0.0039 of proteose B, 0.0012 of albumin and 0.0023 grams of glutelin. Fifteen per cent of Bernton's cases were found sensitive to albumin only, 21 per cent to proteose A only, 21 per cent to proteose A and albumin, and 42 per cent to proteose A and B and albumin.

been well done. The descriptions of Councilman and Lafleur, written in 1891, remain the classic on the subject. The facts laid down by them have been further amplified by Doptei, Kuenen, Christopherson, and recently by Callender. There are, however, facts that bear repetition and seem particularly worth emphasizing after experience in the tropical climate of Egypt where amebiasis is common, and in America where an understanding of the disease is uncommon.

In the American Hospital at Tanta, which cares for both foreign and native patients, 27 per cent of the patients treated in the past three years have had clinical amebic dysentery. This does not represent the number of patients harboring the parasite. The incidence of amebiasis, by which is meant demonstrable infection with *Endameba histolytica*, is very high among the native people. Adequate surveys to show just how high have not been made, but the incidence is certainly so high as to be almost universal among the native population.

The small number of patients suffering from definite disease due to this infection with *Endameba histolytica* is only explainable by a clear conception of the life history of the parasite and the factors concerned in its invasion of the host. The ideal condition for the parasite is a nice adaptation to the host so that there is no disturbance of function which will bring the defense mechanism of the host into play against the parasite. The accepted story of the life of the *Endameba histolytica* shows how this can be. The normal habitat of *Endameba histolytica* is the human colon. The active part of its life cycle is entirely confined here. Dissemination to other hosts occurs in a resistant inactive form, the cyst. The active vegetative organism loses its activity, decreases in size, and forms about itself a resistant capsule. In the encapsulated state, the nucleus divides and subdivides to form four smaller nuclei. In mature cysts there are always four nuclei and very seldom, probably never, more. (Dobell.)

The cysts are found in the normal or nearly normal stools of persons who have recovered from dysentery or who may never have had any symptoms. The cysts are easily injured by drying, heat, cold, or sunlight. Conditions in Egypt and other tropical and subtropical countries are such that transfer of cysts to fresh food materials is common. Chief among the agencies of transfer are hands, water, and flies.

An illustration or two will make this clear. The Mohammedan is required by religious law to wash himself after defecation, not only externally but also the lower portion of the rectum. Because of this fact, the bank of a stream or canal is the most convenient latrine. Water in the canals where there is a dense population is commonly grossly contaminated. Since the washing is performed with the bare hand and fingers the nails especially are grossly contaminated with the person's own feces. Those who are so contaminated and act as food merchants or cooks are constant sources of food contamination.

In tropical and subtropical countries, the water is taken directly from the canals for irrigation and for use in the homes. Fresh vegetables which may

be eaten uncooked are fertilized with night soil, irrigated with canal water and finally kept fresh in market by dipping in canal water

Flies abound in all climates especially in the tropics and where fecal matter containing cysts is exposed they feed upon it. It has been proved that the cysts of *Endameba histolytica* survive in the intestines of the fly and pass through it unchanged (Warron and O'Connor 1914 1916). If the cysts are deposited by the fly on human food they readily find access to the gastrointestinal tract. It has been proved that only mature cysts are infective when eaten (Walker and Sellards). Under conditions existing in the small intestine the cyst wall ruptures and a living ameba escapes. It is generally believed that one organism containing all four nuclei emerges and that this then divides to form four new amebas. No sexual phase has been demonstrated.

As soon as the young parasite comes in contact with the mucosa of the large intestine it attaches itself to the living epithelial cells. Probably by a process of cytotoxicity a superficial erosion of cells is produced and the parasite lives in and upon the dissolved cells penetrating downward as the process of cytotoxicity continues and living in the cytolyzed material close to the living cells. As the parasite invades new tissues the host endeavors to limit and repair the damage done. Usually a state of equilibrium is reached early and the ulceration never becomes extensive. If the lesions are very mild, no subjective or objective symptoms may be noted and the individual becomes a contact carrier. The cysts from his stool are fully virulent and capable of producing severe clinical disease in a suitable host.

The initial infection often leads to a slightly more extensive ulceration of the intestine and the symptoms of diarrhea or dysentery supervene. The attack is quickly overcome and the person becomes symptom free. There are still however cysts in the stool and there may be more or less frequent return of symptoms depending on such things as indiscretion in diet, work, exercise, or exposure. This condition is known as the convalescent carrier (Walker and Sellards). The contact carrier condition is frequently encountered among the native people of Egypt and is seen among foreigners. The convalescent carrier condition is the common condition among the latter.

When a condition of equilibrium between host and parasite has been nearly reached and dysenteric symptoms have abated some of the amebas in the tissues become inactive. Lost by egestion the engulfed red cells and other food in the cytoplasm decrease in size and are discharged from the ulcers into the lumen of the gut. This is the precystic form. A resistant capsule is formed about the parasite as it continues to diminish in size, its nucleus divides and redivides to form four smaller nuclei. Chromatin bodies may form in its cytoplasm, and it is now known as a cyst. In the cystic form the parasite passes from the colon of its host, and the cycle is completed.

Evidence shows that if the parasite passes from the colon of its host before the cysts are fully formed or in the vegetative condition they quickly perish and therefore cannot infect a new host (Walker and Sellards). It is only when the fecal stream through the bowel is nearly or altogether nor-

mally prolonged that there is time for the formation of cysts. In kittens where a severe fatal dysentery is the result of infection, cysts do not appear, and in the liver, lungs, and other tissues, cysts are not found.

If these facts are kept in mind it will be seen why the search is for cysts or precystic forms in the normal or nearly normal formed stool, and for amebas in the dysenteric stool and in the tissues.

It is not within the scope of this paper to discuss the differential characteristics of the vegetative precystic, and cystic forms of amebas. The reader is referred to the abundant literature on this subject.

Apparently all strains of *Endameba histolytica* are equally virulent, but among a number of persons exposed to a given source of infection, a comparatively small number will have clinical evidence of the infection, and the remainder will show only slight signs or none at all. It is a common experience in the tropics to find people showing no symptoms, yet having cysts in their stools. In postmortem work where the possibility of mild to insignificant lesions is kept in mind, a considerable number of amebic ulcerations are discovered in the colon from patients who have died from other causes. Reviews of seven cases that occurred in Pittsburgh and Cleveland showed all to be very severe with overwhelming lesions. Failure to discover milder infections, in the light of experience with the disease in the tropics, suggests the possibility that many mild to insignificant amebic lesions are overlooked in our northern hospitals. Certainly this is true clinically, for of the seven cases all of which were severe and four of which were complicated by liver abscesses, only two were diagnosed clinically and one other suspected.

Where there is an infection in the colon, the ulceration may extend as a superficial erosion or as a deep penetration of the tissue. The former exists very early and is limited in extent. The latter is the common and more typical lesion. The cytolytic activity of the parasite results in a deep, well-like necrosis of the mucosa, and the parasites migrate down through the necrotic material and lie in close proximity to the living cells below. The muscular coats, first the muscularis mucosa and later the muscular wall of the colon, offer resistance to the progress of the lesion, and the ulceration spreads laterally under the mucosa, forming small localized lesions characterized by a narrow perforation of the mucosa communicating with a crater in the submucosa. This is the "bouton de chemise" lesion of the French writers. Grossly, it looks like a yellow, necrotic, slightly raised point, ranging from a pinhead to a pea in size, and located in the mucosa of the colon. Such a lesion might be mistaken at a casual glance for a caseous tuberculous nodule. On section, it shows a narrow orifice, undermining crater, a central more or less necrotic jelly-like mass, very slight surrounding inflammatory reaction, and amebas. This lesion may be the only one present to gross inspection, or it may be, and often is, seen in connection with more advanced lesions in the severely ulcerated colon. As the lesion extends, it increases in size and may penetrate the muscular coats. The amebas advance with the necrosis and remain in large numbers near or sometimes penetrating the cells of the living tissues. If the ulceration is progressive, several amebas may be found in the blood vessels and lymphatics supplying the involved area of

gut. Secondary bacterial infection soon occurs from contact of the ulcers with the fecal stream. The injured cells become more definitely necrotic, an inflammatory reaction sets in and the clinical picture of dysentery is present. The undermined mucosa is cut off from its nutrition, dies and is cast off as sloughs, leaving ragged ulcers with overhanging edges, necrotic bases and surrounding inflammatory reaction. As the ulcers enlarge, they become confluent, first by the formation of submucosal tunnels, and later from the death of the overlying mucosa by channels of ulceration. The intestinal mucosa is pitted and grooved in various sized and shaped areas of ulceration separated by islands and isthmuses of mucosa. The ulcers may penetrate the muscular coats of the intestines and the serosa allowing the contents of the gut to escape into the abdominal cavity. As a rule, however the process is not so rapid and inflammatory reaction about the ulcerated area produces thickening of the gut wall by fibrous tissue and adhesions to surrounding tissues. The bladder and genital tract have been invaded by extension from an intestinal focus.

The ulceration may spread so rapidly in the intestinal mucosa as to cause the overhanging mucosa to fall away from the submucosa before it has had time to become necrotic. Hemorrhages occur from eroded vessels and the blood forms cobweb like clots in the ulcers which give the gut a shaggy appearance. Such was the appearance of the colon of an elderly man who went on a world tour for the sake of his health contracted amebic infection in the Orient and died in Pittsburgh this winter. It represents what may be expected in amebic infection when the reparative powers of the host are at a low level. In another patient in Pittsburgh whose resistance was lowered, 24 centimeters of the colon were completely gangrenous. Still another case of very severe ulceration and gangrene occurred in a native Egyptian who was brought to the hospital because of a fracture of the spine in the upper dorsal region. At the time of admission there were no dysenteric symptoms. There was complete paralysis of the voluntary muscles on both sides below the level of the fractured vertebra and the patient rapidly developed a most dramatic amebic dysentery and died with a gangrenous intestine. Just what part is due to the amebic infection and what to secondary invaders in cases like these is impossible to say. Certainly the widespread destruction would not have occurred without amebas.

The earliest lesions of amebiasis are found in those portions of the colon where stasis is most likely to occur. Cecum, rectum, sigmoid colon, splenic flexure, and hepatic flexure show ulceration in diminishing frequency as named (Chile). In severe cases the colon is likely to be involved throughout its length. The appendix is commonly involved in cases where there are extensive lesions in the cecum. Amebic lesions of the appendix were not encountered in a review of 24 appendices (13 from natives), which were brought from Egypt.

In the primary lesion of the collar button type with jelly like necrotic slough amebas are easy to find. Such lesions may be found alone or in conjunction with others and are to be sought and sectioned. When the ulcers become infected with bacteria amebas are found less numerously. They are

found in the deeper parts of the necrotic base in close proximity to the living tissue, or still more often in areas of necrosis under the overhanging edges of ulcers. Amebas may be seen penetrating among the living tissue cells in the margins of the ulcers or in small blood vessels near them. They occur in groups, are discrete from the tissue cells, and are usually surrounded by a clear zone. The more rapid the process of ulceration and the more violent the dysentery, the more numerous the parasites. Fewer and fewer parasites are seen in lesions as the ulcers become less progressive, and healing becomes evident. Neither in the sections from Egypt nor in cases in America have amebas been found in the presence of granulations. They may be found in ulcers that show healing, but there is no healing in the portion of the ulcers where the amebas are found. The colon of a nurse who had amebic dysentery (diagnosed in Pittsburgh and treated vigorously with emetin before death) showed very extensive ulcerations of the type expected in chronic amebiasis, with fibrous thickening of the gut wall. Grossly there was much evidence of epithelialization of the ulcerated areas, and microscopically there was much granulation tissue. Many sections were made but no definite amebas were found.

Often extensive ulcers may show few amebas after secondary bacterial invasion has occurred and the walls of the intestines have become filled with inflammatory exudate. This may be because the ulcers started by the amebas have been spread more rapidly by the secondary invaders, and the inflammatory reaction exerts an unfavorable influence on the amebas. It is certain that the extent of the ulceration appears at times to be out of all proportion to the number of amebas present and careful search is necessary to bring them to light.

It has been pointed out (Callender) that the type of ulceration seen in an intestine or the type of cells in a stool are not always trustworthy guides in the diagnosis of dysentery. Bacillary dysentery may coexist with amebic. The bacillary picture may be superimposed on the amebic picture. Conversely the finding of amebas in an ulcerated gut does not necessarily prove that the clinical manifestations of disease are due to its presence. Neither does the typical superficial ulceration of bacillary dysentery and the recovery of an organism mean that search for masked ulcers of amebic origin is unnecessary. Similarly the finding of vegetative or encysted amebas in a stool does not necessarily mean that the patient suffers from amebic dysentery.

The character of the stool in amebiasis depends very greatly on the extent of the ulceration, the position of the ulceration high or low in the colon, and upon the type of secondary bacterial invaders present. As a rule, the stool is foul, mixed with feces and swarming with organisms. It is likely to be small in amount in severe cases and may be largely mucus. As the condition of the patient improves, the stool becomes more fecal in character, of a pea-soup consistency, brown in color and containing considerable quantities of blood and mucus. The mucus is found as small masses of sago like material stained with blood and floating in the liquid feces. In very acute cases showing such stools a loop full of feces is always searched first for motile vegetative amebas. It is spread on a glass slide and pressed out

under a cover slip and examined at once. The more acute the ulceration in the bowel, and therefore, the more acute the disease the shorter is the time that elapses between leaving the host's tissue and examination, consequently the better the chance of finding the organism motile. If the ulcers are healing, or partially so, and the host is resisting his infection the diarrhea will be less violent, the chances for the organism to begin the process of encystation will be greater, and many of the amebas will become inactive, and even though kept under ideal conditions on a warm stage it may be difficult to detect motility. Only motile active amebas ingest red blood cells, and so this diagnostic sign should not be expected under conditions which produce precystic and cystic forms.

In diarrheic stools or formed normal stools such as are obtained from convalescent carriers with a small amount of ulceration in the intestine many precystic forms and cysts will be found in the stool. If the stool has on its surface a trace of blood or a bit of bloody mucus this should be especially searched. The specimen is macerated with a drop of normal saline on a glass slide and examined unstained with the low power objective against a dark background. Amebas, cysts and precystic forms appear as bright, rounded, translucent bodies of uniform or irregular size, depending on whether they are in the same stage of development. No time is wasted looking for motility unless the condition of the patient and character of the stool would indicate that the disease is acute or very active.

To bring out the cysts and make it possible to count their nuclei, the fecal suspension is mixed with a watery solution of iodine in potassium iodide 'the stronger the better' (Dobell). A drop of strong tincture of iodine may be used if it is put on the slide first and allowed to evaporate almost to dryness. The drop of emulsion of feces is then rubbed up in the center of the iodine area.

To examine the nuclear structure of amebas and cysts fixation of perfectly fresh specimens while they are still wet is necessary (Dobell, 1919).

Amebic dysentery is an unfortunate event from the ameba's point of view. The organisms are forced into the outer world out of their normal habitat so suddenly that they have no time to encyst themselves. Just as unfortunate from the point of view of the parasite is the formation of secondary lesions in other parts of the host than the colon because here again cyst formation does not occur and there is no possibility of completing the life cycle.

The most common secondary lesion is liver abscess. The intestinal ulcers furnish the atrium of infection and amebas pass by way of the blood stream to the liver. The first evidence of their presence in the liver is a hepatitis then small abscesses form. Probably at first they are multiple, and although usually in the right lobe they may occur in any part of the liver. The small early lesions contain a central, nonpurulent necrotic mass and many amebas. The abscesses increase in size due to the action of the parasites and coalesce to form larger abscesses. It is not until the abscess reaches a considerable size that it can be diagnosed clinically. This may be the reason that some authors have described liver abscesses as usually solitary. Experience has

taught that in many cases they are multiple. As the size of the necrotic area increases, the abscess content is likely to be invaded by bacteria which change its character, giving it a purulent nature. Rupture of blood vessels in the necrotic walls allows the escape of blood into the cavity and imparts to the mixture the characteristic anchovy sauce appearance.

The character of the wall of the abscess depends somewhat upon the course and duration of the lesion. If the abscess is recently formed, the wall will consist of a necrotic zone of tissue gradually passing over into living tissue cells. In abscesses of purely amebic origin there is necrosis of tissue without pus formation. The amount of inflammatory reaction in the wall depends on the character and presence of secondary invaders. If the progress has been slow or kept at a standstill by treatment or by the resistance of the patient there will be plentiful fibrosis in the abscess wall.

In amebic abscesses that are increasing in size, the wall is filled with smaller secondary abscesses which contain an abundance of amebas. As the smaller abscesses increase in size, they coalesce with the main abscess and become part of it. The result is that the inside of the amebic abscess has an irregular sloughing wall with many larger and smaller cavities opening into it. The microscope shows multiple small early and larger later abscesses in the zone about the main abscess. Amebas are found in all abscesses but are most numerous in the smaller ones and always near the living tissue.

In old, long standing, secondarily infected and treated abscesses, amebas may be hard to find. When they are few, great care must be exercised not to confuse large tissue cells occurring singly with amebas. The ameba does not normally contain fat, so fat stains help to differentiate them from the large vesicular fat-bearing cells that are sometimes seen packing the spaces between fibrous tissue bands and penetrating among stroma cells near chronic abscesses. Sections carefully stained with iron hematoxylin after fixation in bichloride solution bring out the differential characters of the nuclei (Dobell, 1919).

The lung may be involved secondarily to abscess of the liver by perforation of the diaphragm and pleura. There is no essential difference between the progress of the abscess in the lung and in the liver.

Abscess in the brain arises, as a rule, from the excitation of operative procedures on abscesses of the liver. It is undoubtedly embolic in origin. No cases of brain lesion have been encountered in this study.

Amebic lesions occur in other regions of the body. They are nearly, if not always, due to extension of a colon lesion to a devitalized area. Infection of the bladder, testes, and seminal vesicles, peritoneum, abdominal wall, and skin have been discovered, but such lesions must be regarded as more or less accidental. Smith records an amebic infection of the knee joint with multiple sinuses. Kofoid and his associates, by a method of their own, claim to have demonstrated amebas in the lesions of chronic arthritis and in the lymph nodes of Hodgkin's disease. Their results have not been generally accepted.

CONCLUSIONS

1 The correct interpretation of an amebic lesion depends upon a clear recognition of the status which exists between the host and parasite, and the secondary modifying factors which may intervene to obscure the picture

2 The fact that amebic infection exists without symptoms or with very minor symptoms and minor lesions often leads to failure in recognizing cases

3 Recognition of cases without symptoms is possible only through search for cysts in the stool. Experience has taught that even then only positive findings are conclusive as cysts may not appear in the stool of carriers over considerable periods of time depending upon the extent of the lesion and whether or not it is progressing or regressing

4 Recognition of cases with dysenteric symptoms consists in the identification of vegetative amebic procystic forms or cysts in the absence of more obvious etiology

5 The recognition of amebic ulceration depends upon the gross appearance only as it is suggestive; the final decision rests on finding amebas in the lesions

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STUDIES OF THE ACTION OF SODIUM THIOSULPHATE IN METALLIC INTOXICATIONS*

I THE EFFECT OF SODIUM THIOSULPHATE ON ARSENIC ELIMINATION

By A G YOUNG, PH D, ANN ARBOR, MICH

IN 1920 Ravaut¹ reported beneficial results obtained by treating arsenical dermatitis by intravenous injections of sodium thiosulphate. This was followed by the work of McBride and Dennie² in this country. Since then the use of this compound has been extended to the treatment of other metallic intoxications, such as those due to lead, bismuth, mercury, etc.³ The work so far consists largely of clinical reports. The general opinion at first seemed to be that sodium thiosulphate increased the rate of elimination of the toxic substance, thereby exerting its beneficial effect. Gioehl and Myers⁴ and Myers, Maples, Gioehl, and Thione¹⁰ report that sodium thiosulphate increased the rate of arsenic excretion in metallic toxemias. Kuhn and Loevenhart⁵ experimenting on rabbits found that sodium thiosulphate reduced the total amount of arsenic excreted per twenty-four hours. Later Kuhn and Reese⁶ reporting on clinical cases said that "The treatment increases the excretion of arsenic and hastens the restoration of the kidneys to normal."

TABLE I
SHOWING THE EFFECT OF SODIUM THIOSULPHATE ON ARSENIC TOLERANCE

CONTROLS				TREATED						
NA ARSENATE				NA ARSENATE		NA THIOSULPHATE				
MC	PFR	KG	LIVED	MG	PFR	KG	MG	PER	KG	LIVED
Rabbit No 13				Rab No 10						
4/23/25		25		4/ 7/25	30		50			
4/29/25		15		4/17/25	30		—			
5/ 5/25	Dead		12 days	4/23/25	30		—			
Rab No 15				4/24/25	Dead					17 days
4/23/25		25		Rab No 16						
4/29/25		15		4/23/25	25		—			
5/ 5/25	Dead		12 days	4/24/25			50			
Rab No 1				4/29/25	15		—			
3/27/25		25		5/ 8/25	15		—			
4/ 1/25		25		5/12/25			50			
4/ 3/25	Dead		8 days	5/15/25	15		—			
				5/16/25	—		50			36 days
				Rab No 17						
				4/24/25	25		50			
				4/29/25	15		—			
				5/ 8/25	15		—			
				5/12/25	—		50			
				5/15/25	15		—			
				5/16/25	Killed					32 days

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These reports are conflicting and suggest the following questions

- Does sodium thiosulphate act differently in man than in rabbits?
- Does it act the same on organic as on inorganic arsenic?

The work herein reported was undertaken in an attempt to answer the above questions

METHOD

Rabbits—Sodium arsenate was administered intravenously to rabbits in doses of from 25 to 30 mg per kg of body weight. Half of each group of rabbits received sodium thiosulphate intravenously in doses of 50 mg per kg at intervals between the arsenic injections as shown in Table I. If the animal survived the initial dose of arsenic a second dose of 15 mg per kg was given. At the time of death the animals were autopsied and examined for gross lesions.

Results—Table I shows the amount of arsenic and thiosulphate given and the number of days the animals survived. Two of the animals receiving no thiosulphate succumbed on the twelfth day after receiving but 40 mg per kg of sodium arsenate in two doses of 25 and 15 mg respectively. The third one survived but two days following a second injection of 25 mg per kg of sodium arsenate. The three animals receiving thiosulphate treatment lived seventeen, thirty-six and thirty-two days respectively, one requiring three doses each of 30 mg per kg to produce death, another required one dose of 25 mg and three doses of 15 mg per kg to produce death. Rabbit No. 17 survived one dose of 25 mg and three doses of 15 mg per kg but had to be killed at the end of the thirty-second day because of an injury. It will be noticed that rabbit No. 10 survived seventeen days after receiving but one injection of thiosulphate.

TABLE II

SHOWING THE EFFECT OF SODIUM THIOSULPHATE ON ARSENIC EXCRETION IN RABBITS

RABBIT	MG NA ARSENATE PER KG	TOTAL AMT ARSENIC GIVEN, MG	URINE ANALYSIS MG AS EXCRETED			MG PER KG NA THIOSULPHATE
			24 HR	42 HR	PER CENT TOTAL AS GIVEN	
1	25	28.39	No urine	31 mg	7.4	None
5	30	27.46	4.42 mg	No urine	16.1	None
6	30	30.09	4.08 mg	No urine	13.2	None
7	30	34.52	4.35 mg	No urine	12.6	None
9	30	17.19	9.91 mg	No urine	19.6	None
					Avg Excr = 12.38%	
2	25	29.96	No urine	2.51	8.4	50
4	25	41.69	3.21	No urine	7.7	50
8	30	20.19	2.08	No urine	10.3	75
10	30	35.39	2.43	No urine	6.9	50
					Avg Excr = 8.17%	

This data shows that the thiosulphate increased the rabbits' tolerance to repeated sublethal injections of sodium arsenate. It is therefore interesting to note that it would not prolong the animal's life if single large doses of the arsenical were given. That is, it does not increase the MLD to a marked extent as shown by the following data.

The MLD of sodium arsenate for rabbits is 45 mg per kg. This dose was administered to a group of five rabbits. The injection was followed im-

mediately by an injection of 50 mg per kg of sodium thiosulphate. Four of the five animals survived while without the thiosulphate it is safe to predict that three or more would have died. A second group of five rabbits was given 50 mg per kg of sodium arsenate followed by the same amount of

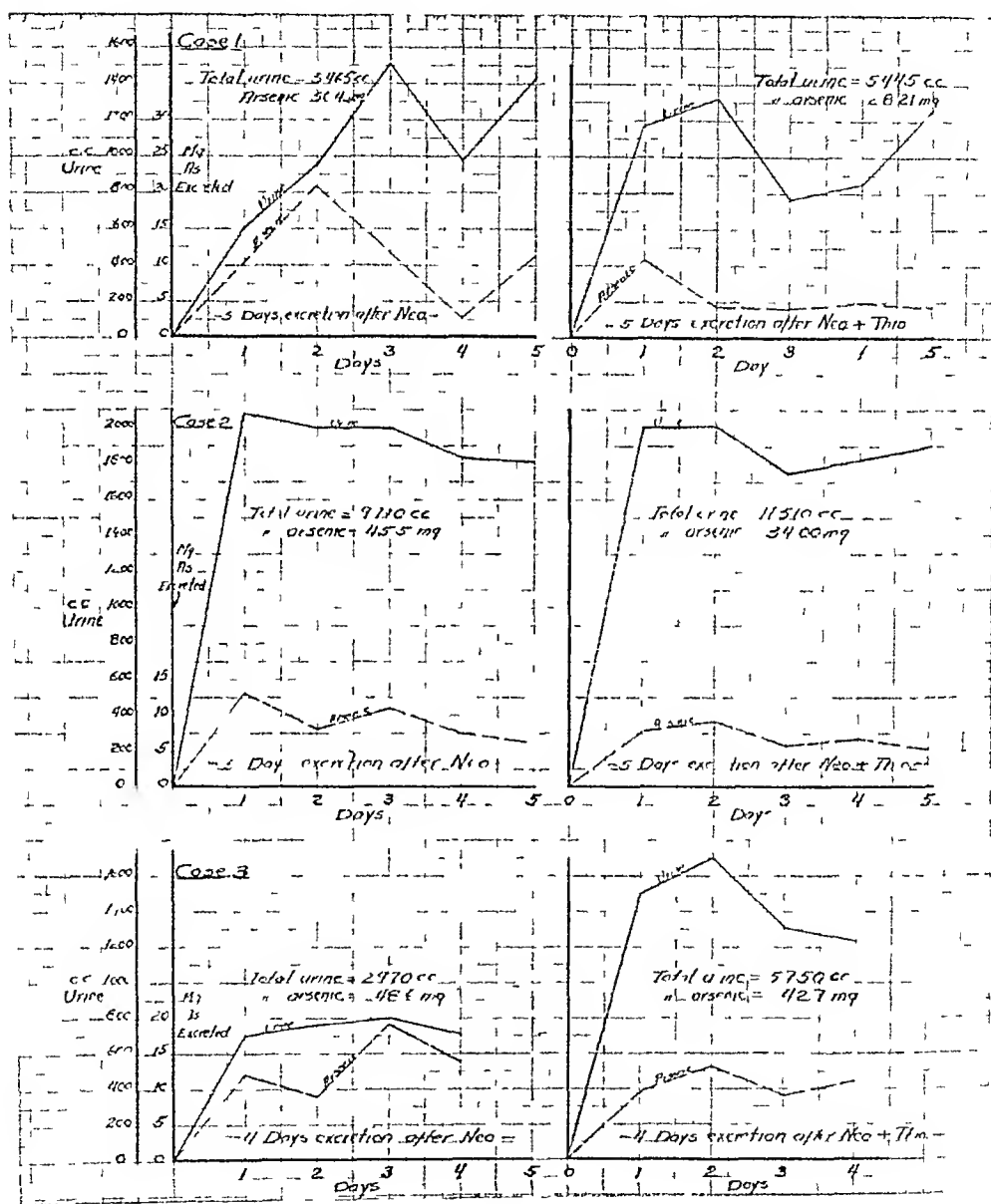


Chart I

sodium thiosulphate. Of this group only one survived. This shows that the thiosulphate was not a protection against an approximate increase of 10 per cent of the MLD. The same results are reported by Voegtlin using sodium arsenite and arsenoxide.⁹ Table II shows the effect of sodium thiosulphate on the arsenic excretion of rabbits. Two groups of animals were used. The

first group received no sodium thiosulphate while the animals of the second group received 50 m. per kg of sodium thiosulphate following the administration of the arsenic. Examination of this table shows that the average arsenic excretion of the animals receiving thiosulphate was 35 per cent lower than the average excretion of the animals receiving no thiosulphate.

This confirms the finding of Kuhn and Loevenhart who reported that sodium thiosulphate reduced the total amount of arsenic excreted by rabbits per twenty four hours.

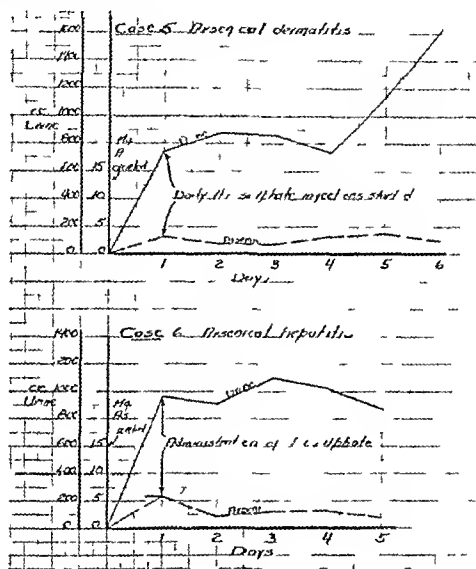


FIGURE 11

CLINICAL INVESTIGATION

In reviewing the clinical literature to date one finds many case reports but few of them are accompanied with quantitative studies of arsenic excretion before and after the thiosulphate was given. In the references quoted above⁶ one finds that a twenty four hour specimen of urine taken previous to the administration of the thiosulphate has been considered indicative of the rate of excretion of the arsenic. Thiosulphate was then given and the urine analyzed for five or six days in succession.

Since the quantity of urine voided from day to day varies considerably with the diet fluid intake, etc. it seemed logical to expect that the arsenic output in cases of intoxication might vary from day to day also. In two cases of arsenical intoxication which I have studied the administration of thio

sulphate was followed by a decrease in the arsenic excretion as determined on twenty-four-hour samples taken before and after its administration. However, since the doubt existed as to the value of such findings the following method was used. Patients receiving arsphenamine or neoarsphenamine but who showed no toxic symptoms were selected after they had received four or five weekly treatments. Following a single treatment, twenty-four-hour specimens of urine were collected for four or five days. Each sample was analyzed to determine the amount of arsenic excreted each day under normal conditions and also to determine the variation in the rate of elimination from day to day. The following week when the patient received the arsphenamine treatment, sodium thiosulphate was given intravenously for four or five days in daily doses of 10 cc of a 10 per cent solution. The urine was collected as before and analyzed. Four cases were studied. Chart I shows the results obtained in three of the cases studied in this way. Examination of this chart shows that in Case 1, approximately the same volume of urine was excreted during each five-day period but that only one-half the amount of arsenic was excreted when the sodium thiosulphate was given. It also shows that the amount of arsenic excreted from day to day varies so much that a single twenty-four-hour sample cannot be considered indicative of the rate of excretion. Case 2 is very similar, showing a decrease of 36 per cent in the arsenic output. In Case 3 the urinary output was more than doubled when the thiosulphate was given, but the arsenic output was practically unchanged. In Case 4 the thiosulphate decreased the arsenic output for the four-day period about 25 per cent, while the urinary output was practically unchanged.

Cases 5 and 6 (Chart II) were cases of arsenical intoxication. It will be noticed in Case 5 that the arsenic output was not materially influenced, yet the patient improved. This is in keeping with Ravaut's report of beneficial results obtained by the use of sodium thiosulphate in dermatitis not due to metallic intoxication. Case 6 shows a definite reduction of the daily arsenic excretion.

DISCUSSION

Examination of the literature shows that sodium thiosulphate is considered by many to be an "ideal" antidote for arsenical poisoning and that most observers consider that its beneficial action is exerted by increasing the rate of arsenic elimination through the kidney. One is certainly convinced that clinical improvement has been seen in a great many cases of intoxication, but so far no one has brought forth conclusive evidence to show how this beneficial result is obtained. Marples and Myers have attempted to explain the action of sodium thiosulphate on the assumption that a hypothetical "barrier" function of the cell has been interrupted. They say: "It may be reasonably assumed that the reason for the effectiveness of sodium thiosulphate in cases of arsenic intoxication is due to a stimulation of the processes which normally take care of arsenic but which have become sluggish on account of the toxic action of the metal." However, no statement is made as to what constitutes the "processes" which are stimulated nor is any evidence offered to prove the presence of a "barrier" function in the cell.

The work of Voegtlin, Dyer and Leonard³ provides a much better explanation of the toxic action of arsenic. They have shown that the toxicity of arsenoxide is greatly reduced by glutathione and a number of other compounds containing an SH group. They say 'It is quite likely that arsenoxide reacts chemically with the reduced form of glutathione and perhaps also with some other SH compounds. * * * Reactions of this type would naturally reduce the concentration of the SH compounds of the cell in proportion to the amount of arsenoxide added. If the amount of arsenoxide furnished to the cell should exceed a certain limit poisoning and death would necessarily follow as a result of the reduction of the absolute amount of SH compounds below the physiologic requirement. After quoting the work of Hopkins and Dixon to show the important part played by glutathione in the respiration of the cell they conclude that 'If glutathione really plays such an important part in the life of the cell, then it is not at all surprising that substances, such as arsenoxide which react with the SH group of glutathione, should exert a toxic action. * * * In the final analysis arsenic would have to be considered as a poison which causes death of the cell by interfering with the oxidative processes governed by glutathione.'

It is surprising that so few of the papers published have shown any attempt to follow the effect of thiosulphate on the rate of arsenic excretion. In the work reporting quantitative results one finds that a twenty-four hour sample taken previous to the administration of the thiosulphate has been considered indicative of the rate of excretion. Kuhn and Reese say 'In some cases the increase in the excretion was marked and in others it was slight. * * * the more marked increases were of slight duration and could not be maintained by subsequent doses of sodium thiosulphate.' On examining the charts submitted by these investigators one is impressed with the fact that the administration of sodium thiosulphate is sometimes followed by a rise and sometimes by a fall in the arsenic elimination. Groehl and Meier⁴ (page 693) submit a chart showing that the urine previous to the administration of the thiosulphate contained 8.18 mg of arsenic. Two days after the thiosulphate injection had been started 31.48 mg of arsenic were found. However, the arsenic output then fell to 4.01 mg. for two days even though the thiosulphate was being given. Further perusal of this chart shows a rise and fall of the arsenic content throughout the period of treatment which does not correspond with the administration of the thiosulphate. Most observers agree that less kidney damage is found after the administration of sodium thiosulphate. Is it not possible that the drug may act in two ways: (1) by forming an insoluble compound with the arsenic which is excreted more slowly and therefore does not reach the kidney in such high concentration and (2) by supplying available sulphur to the cells?

CONCLUSIONS

From the data submitted here one can conclude that in most instances the thiosulphate has markedly diminished the rate of arsenic excretion and has never increased it even when there is pronounced diuresis as in Case 3.

The experimental work on rabbits would indicate that the drug has some value in preventing kidney damage in chronic intoxication but that its value as an antidote when large amounts of arsenic have been ingested is questionable

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STUDIES OF THE ACTION OF SODIUM THIOSULPHATE IN METALLIC INTOXICATIONS

II THE EFFECT OF SODIUM THIOSULPHATE UPON THE EXCRETION OF LEAD*

By ARTHUR C. CLARK, M.D. AND A. G. YOUNG, PH.D.

INTRODUCTION

SINCE the work of Ravaut,¹ numerous investigators have used sodium thiosulphate in varying concentrations, both intravenously and by mouth, in the treatment of lead intoxications. Beneficial results have been claimed from its use. The mechanisms by which these effects occur have not been determined, though it has been inferred that sodium thiosulphate either converts an insoluble lead compound into a soluble, nontoxic, thiosulphuric acid derivative, or of a complex lead proteinate², or that it converts the soluble toxic metal into an insoluble, nontoxic compound.³ If either premise is true, there should be some evidence of change in the amount of lead excreted in the urine and feces of a lead-poisoned animal after the administration of sodium thiosulphate. The investigation was devised to obtain an answer to this question.

It has been shown by Aub, Fairhall Minot, and Reznikoff that lead, whether administered by mouth insufflation or intramuscularly, is probably carried in the blood stream as a colloidal phosphate. Colloidal lead phosphate is then brought into contact with every body tissue, but marked retention occurs only in bones where it is deposited, under favorable conditions, as the tertiary phosphate. This latter compound is very sensitive both to changes

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in the H ion concentration and to the blood calcium. Lead phosphate is released into the circulation when the blood calcium is low or when there is a state of acidosis or alkalosis. It remains stored when the blood calcium is high and the blood P_H normal. Lead is excreted by the gastrointestinal tract, kidney, bile, and skin, but the greatest excretion is in the feces.

It is, therefore, evident that diets high in absorbable calcium and low in acid precursors would tend to increase the storage of lead. Whereas, diets which are low in absorbable calcium and which are acid producing, would tend to increase the elimination of lead. It is then possible to compare the effect of sodium thiosulphate upon the excretion of lead when the diet is high in calcium and low in acid precursors or when the diet is low in calcium and high in acid producing substances.

METHOD

Guinea pigs, rabbits, and white rats were used. They can be conveniently dealt with under three headings: A, B and C.

The animals of Group A consisted of two guinea pigs. The animals of Group C consisted of five rats. The animals of Group A were fed a diet in which was incorporated small amounts of lead acetate. This diet was fed until each animal had ingested one hundred and fifty milligrams of lead acetate. The animals of Group C were fed a similar diet in which lead acetate was present in small amounts. The rats received this diet until each animal had eaten two hundred and two milligrams of lead acetate. All animals were then fed a high calcium lead free diet for ten days to allow any lead present in the gastrointestinal tract to be excreted and to aid in storing the lead present in the circulation. This diet consisted of lettuce, milk, calcium lactate as a 1 per cent solution in milk, and bread. The animals of Group B consisted of two rabbits. Each was poisoned by a subcutaneous injection of 400 milligrams of lead acetate. All the animals of Groups A and B were put into separate metabolism cages, the bottom of each cage having been previously paraffined so that possible contamination from any lead present in solder would be avoided. All the animals of Group C were put into one metabolism cage and the urine and feces collected together under similar precautions used with the animals of Groups A and B.

Three day specimens of urine and feces were collected and analyzed together for lead by the chromate method and by Fairhall's direct precipitation method.⁶ During the period when the excreta were being collected for analysis of its lead content, two general types of diet were used. The first diet consisted of oatmeal, corn meal, and water. Table I shows such a diet to be low in calcium and high in acid precursors. The second diet consisted of milk and corn meal. Table I shows such a diet to be high in calcium and to have an excess in basic properties. A few leaves of alfalfa were given to all animals each day.

All animals were first fed diet No. 1 for a period of three days. The urine and feces were then collected and the cage was cleaned. Following this they were given diet No. 1 plus 0.5 gram of sodium thiosulphate per kilogram of body weight dissolved in their drinking water. At the end of

TABLE I

THE CALCIUM OXIDE AND PHOSPHORUS PENTOXIDE CONTENT OF MILK, CORN MEAL, AND OATMEAL EXPRESSED IN PER CENT PER 100 CALORIES*

FOOD	CaO	P ₂ O ₅	EXCESS ACID	EXCESS BASE
KIND	PER CENT PER 100 CALORIES	PER CENT PER 100 CALORIES	GRAMS	GRAMS
Milk	0.456	0.599		5.0
Corn meal	0.004	0.080	1.5	
Oatmeal	0.030	0.216	3.0	

*Sherman

TABLE II

THE EXCRETION OF LEAD DURING PERIODS OF THREE DAYS ON DIETS COMPARATIVELY LOW IN CALCIUM AND HIGH IN ACID PRECURSORS, HIGH IN CALCIUM AND LOW IN ACID PRECURSORS, AND WITH ADDITION OF SODIUM THIOSULPHATE TO EACH DIET

GROUPS	ANIMAL	DIET NO. 1		DIET NO. 2	
		LOW CALCIUM ACID FORMING	SAME DIET PLUS SOD. THIOSULPHATE	HIGH CALCIUM LOW ACID	SAME DIET PLUS SOD. THIOSULPHATE
	Number	Mg. of lead	Mg. of lead	Mg. of lead	Mg. of lead
A	Guinea pig No. 1	1.456	1.414	0.928	Lost
	Guinea pig No. 2	2.277	1.259	0.310	{ 0.310* 0.690
B	Rabbit No. 5	1.380	1.367	{ 1.311* 1.194	0.897
	Rabbit No. 8	1.242	0.745	trace	trace
C	{ Rats Nos. 1, 2, 3, 4, and 5	{ 0.811* 1.187	0.828	0.345	0.552

*Determinations done on different three-day periods

a three-day period the urine and feces were again collected and the cage was cleaned and reparaaffined. All animals were then placed upon diet No. 2 for a three-day period and the urine and feces collected. They were then placed upon diet No. 2 plus the addition of 0.5 gram of sodium thiosulphate dissolved in milk. The urine and feces were collected at the end of three days on this diet. All three-day specimens were analyzed together for their content of lead.

DATA

It will be observed from Table II that the two animals of Group A excreted more lead on the first diet than they did on the second diet. The decrease produced by diet No. 2 amounted to 43 per cent in animal No. 1 and 86 per cent in animal No. 2. When sodium thiosulphate was added to diet No. 1 there was no appreciable change in the excretion of lead in one animal and a decrease of 50 per cent was noted in the other animal.

Rabbit No. 5 of Group B, was pregnant while on Diet No. 2, and we feel that the results obtained during this period are not representative of the lead which might have been excreted had she not been pregnant, inasmuch as her calcium reserve would be very much depleted while the embryos were

developing and her blood P_n would tend to be more toward the acid side. These conditions more closely simulate those produced by the low calcium and high acid diet. Likewise, there is no appreciable change in her lead excretion while on diets Nos 1 and 2. When sodium thiosulphate was added to the first diet, no effect was observed but when added to the second diet the lead excretion fell approximately 25 per cent. Animal No 8 showed a marked decrease in the excretion of lead, when shifted from the low to the high calcium diet the excretion fell about 40 per cent. The addition of sodium thiosulphate to the high calcium diet caused no change.

Group C shows approximately the same changes observed in Group A and Group B. There is a marked decrease in the lead excretion on diet No 2 compared with the excretion of lead on diet No 1. When sodium thiosulphate is added to the first diet there is a slight decrease in the lead excretion and when sodium thiosulphate is added to the second diet there is a slight increase in the amount of excreted lead.

DISCUSSION

It will be observed from the results compiled in Table II that diets which are low in calcium and comparatively high in acid precursors tend to favor the excretion of lead. Diets which are high in calcium and low in acid precursors tend to favor the retention of lead. Our work then confirms the work of Aub, Fairhall, Vivot and Reznikoff in this respect. The diets used in this experiment were not extreme in either their calcium content or their acid properties and consequently the lead excretion was not great.

The addition of sodium thiosulphate to diet No 1 seemed to lower somewhat the excretion of lead. We feel these results arise from the fact that sodium thiosulphate is an alkaline salt which when added to a diet would tend to decrease the acidity of the diet if the diet were potentially acid or it would tend to increase the alkalinity of the diet if the diet were primarily alkaline. Diet No 1 is an oat diet which has been shown by numerous investigators to be acid producing.⁶ Theoretically the addition of sodium thiosulphate would tend to decrease the acidity of such a diet and would tend thereby to favor the retention of lead by the animal. Diet No 2 is also an oat diet, but milk is present also which is potentially alkaline. These two factors would tend to neutralize each other. Sodium thiosulphate added to the second diet would tend to increase the alkalinity and favor lead excretion. It will be noted that the excretion of lead is greater on a diet high in calcium to which has been added sodium thiosulphate than on a similar diet without sodium thiosulphate. The difference however, is minor when compared to the amount of lead excreted on a low calcium high acid diet.

The sodium thiosulphate was given in large doses by mouth to animals which had previously been fed lead but which at the time they were studied had only absorbed lead present. They, also were free of symptoms of lead poisoning when the drug was administered so that the effect of sodium thiosulphate either upon the symptoms of lead poisoning or its effect when given to animals with ingested lead still present in the gastrointestinal tract is not determined.

SUMMARY

A review of the literature shows that no one has subjected the effect of sodium thiosulphate on lead intoxications to a carefully controlled study. Symptomatic improvement has been looked upon as evidence of the beneficial effect of the thiosulphate treatment. The recent work of Aub, Fairhall, Minot, and Reznikoff⁷ has shown that substances tending to reduce an acidosis or alkalosis in cases of acute lead poisoning produce symptomatic improvement. They have also shown that diets high in calcium create a storage of lead in the bones and a rapid disappearance of all symptoms. One is impressed by the fact that no reference is made to the diet of patients being treated with sodium thiosulphate for lead intoxications when certain diets have been shown to play such an important part in the alleviation of symptoms. This omission alone is sufficient to cast considerable doubt on the value of the clinical evidence submitted to show that sodium thiosulphate is an antidote for lead poisoning.

In view of the work presented here and the lack of evidence to substantiate the claims made, we feel that there is reason to doubt the value of sodium thiosulphate as an efficacious antidote in lead poisoning until further study shows it to be advantageous. Our results would indicate that any other alkaline drug might give the same results as we obtained with sodium thiosulphate.

CONCLUSIONS

1 Our results confirm the work of Aub, Fairhall, Minot and Reznikoff, that diets which are high in calcium tend to decrease the excretion of lead, and diets which are low in calcium and acid-forming tend to increase the elimination of lead.

2 Sodium thiosulphate given by mouth in doses of 0.5 gram per kilogram of body weight to guinea pigs, rabbits, and rats previously poisoned by lead does not appreciably affect the excretion of lead.

3 The slight effect exerted by sodium thiosulphate upon the excretion of lead is due to its alkaline reaction and not to any ability to form soluble or insoluble lead compounds.

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ROLE OF SPECIFIC AND NONSPECIFIC FACTORS IN ALLERGY AND ALLERGIC EQUILIBRIUM*

BY WARREN T. VAUGHAN, M.D., RICHMOND, VA.

SUCH terms as protein sensitization, anaphylaxis, immunity and allergy have come into such general use that we are prone to assume an understanding of the fundamental processes in these conditions which is not justified by the facts. Many theories have been advanced to explain the phenomena of anaphylaxis and allergy, a great proportion of which have already passed into the discard. Even those theories which have come nearest to explaining all of the phenomena observed have met with the obstacle of new observations, unknown at the time at which the theory was proposed, observations which must perforce be fitted into the picture.

With the development of new methods of study, old experiments are repeated, we might say with different instruments or measures, and new light is shed upon their mechanism. We formerly discussed immunology in terms of chemistry, later in the nomenclature of physical chemistry, and now from the point of view of the colloidal chemist. The biologic processes which we are attempting to study remain unchanged. It is our methods which vary, and unfortunately with the development of any radically new method of investigation a new terminology is almost perforce introduced into the medical vocabulary, with the result that a single phenomenon masquerades often under many names.

The language of immunology is complex and vague chiefly because of our lack of knowledge of the processes involved. Without this knowledge we cannot use more exact terms. The immunologist 'struggles with agents of unknown composition and measures results with a yard stick of uncertain accuracy. The physical chemist applies methods of greatest accuracy to materials of uncertain nature and to reactions which are modified by an almost infinite number of unknowable variables.'

Our lack of clear understanding of the processes of anaphylaxis and allergy is a decided handicap. As Dean has well said "Ignorance however aptly veiled in an attractive terminology remains ignorance."

Theories are but the stepping stones of our progress toward knowledge. Without them we could not progress, nor must we remain too long upon one stone. Each theory has its temporal value but it is deeply rooted in its own environment and we must be searching for new ones farther along in the path of progress. Some stones are larger than others and on the basis of a single theory we often cover rather a surprising distance. The end of the stone will, however, ultimately be reached, and we may be happy in the assurance that a new one will be found with a strong enough base for us to travel onward.

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It would appear that in the realm of immunology we have in recent years been traversing a surprisingly large stepping stone. Recent studies, especially in colloidal chemistry, have raised questions which the older theories of anaphylaxis and immunity appear unable to solve, and yet those theories have been the basis for all of our knowledge of clinical allergy as we see it in hay fever, asthma, urticaria, and several other conditions which I shall discuss later. Whether the theories have been true or false, they have been instrumental in aiding or relieving millions of suffering humanity. Whatever their future status, in their own times they were as near to the truth as could be approached.

In a paper on new viewpoints in allergy we must presume an understanding of the older viewpoints. For the sake of orientation, therefore, I shall review very briefly one theory of anaphylaxis, on the basis of which much of our present knowledge is builded, a theory which will almost necessarily require modification both in terminology and in context as the result of accumulating information, but one for which, so far as I know, no completely satisfactory substitute has been proposed. I refer to the explanation of anaphylaxis proposed by Victor C. Vaughan.²

It appears to be a biologic law that when a living cell comes in contact with a foreign protein, it will endeavor to elaborate a ferment or enzyme which will digest and destroy the protein. If no enzyme already exists for the particular protein, the cell will endeavor to manufacture one. The primary reason for this digestion of a foreign protein in the environment of a cell is of course the acquisition of food material. Even in the human body this is equally true as far as the individual cell is concerned and the process would be as harmless as it is to the unicellular organism were it not for the fact that poisonous decomposition products are liberated, not in the outside environment but within the human economy itself.

When a foreign protein is introduced directly into the body, it will continue to exist as such until the body cells have elaborated an enzyme or antibody capable of digesting and destroying that protein. Whether it be a living microorganism or unorganized, as serum or egg albumen, it will persist in the blood, and, if living, may multiply, until sufficient enzyme has been produced to digest and destroy it. A good time, therefore, for positive blood cultures in typhoid fever is during the incubation period, before the symptoms of the disease have become manifest. Longcope has shown that horse serum injected into rabbits continues to exist as such until after the development of serum sickness.

Normally, the body tissues are not provided with specific ferments or enzymes for those proteins which should have been digested before absorption from the intestines. Once having acquired the ability to digest a protein, however, the cells retain that power, and can reproduce the specific enzyme whenever stimulated by the presence of that protein. From ten days to two weeks is usually required for the original elaboration of the antibody, but thereafter the introduction of the protein calls forth an almost instantaneous production.

Every protein appears to consist of a poisonous and of a nonpoisonous portion. The poisonous portion of the molecule is in all probability similar in all proteins. The nonpoisonous portion is different for every protein, and on this difference rests the specificity of the protein. The newly formed enzyme digests away the specific nontoxic portion of the molecule thereby liberating the poisonous fraction. Symptoms of protein intoxication result from the liberation within the body of the poisonous portion. While the tissues are first learning to produce the antibody protein is split slowly so that at no one time are large amounts of poison present and the symptoms are either absent or very mild. However mild symptoms may follow even the first injection of foreign protein as in the familiar cases of serum sickness. The slow liberation of poison prevents any serious reactions.

If, after the body cells have become sensitized—have learned to pour out rapidly the new destructive antibody—the antigen or foreign protein be again administered parenterally the high resultant concentration of antibody produces such rapid destruction and liberates such large amounts of poison in a short time, that serious symptoms result. In this way typical anaphylactic shock may be produced. The symptoms are not necessarily respiratory. In the guinea pig anaphylactic death is respiratory and the symptoms are of asthma. In the dog they are chiefly circulatory. All parts of the body are involved in anaphylactic shock and the symptoms may vary accordingly.

In typhoid fever when the tissues have at length become sensitized, the growth of the bacilli in the circulation has already produced such large amounts of protein to be digested that serious symptoms ensue. In malaria the extrusion of the plasmodia from the red cells into the plasma, where digestion rapidly occurs gives rise to the characteristic rigor and high fever.

In this paper I shall not enter into the controversy as to whether the mechanism of clinical allergy is identical with that of experimental anaphylaxis or whether it is an analogous but basically different reaction. It is difficult to explain some of the facts of clinical allergy in terms of existing theories of anaphylaxis but nothing has been conclusively demonstrated either way and it is possible that some future stepping stone will be found which will reconcile the points of apparent disagreement.

Meltzer in 1910 first suggested that bronchial asthma may be an anaphylactic phenomenon. If this be true we must presume that at some time the asthmatic patient first became sensitized by the penetration of the allergenic foreign protein into the body and that it was not until after this that the reintroduction of the same protein produced the symptoms of allergy. The objection has been raised especially by Coca² that we have been unable to prove the primary sensitization and that allergy is more often an hereditary manifestation. He also raises the objection that allergy has not been proved an antigen-antibody reaction that antibodies have not been found in the serum of allergies. There is no denying the hereditary factor in allergy, and it is true that some infants are born sensitized to certain proteins. The most recent work in this field has been contributed by Balcer¹⁷. So far no analogy to this has been observed in experimental anaphylaxis. Passive

anaphylaxis may be transmitted from mother to offspring in guinea pigs, but the sensitization dies out in the progeny in a few weeks

On the other hand, Coca's insistence upon the presence of an antigen-antibody reaction is, as Kolmer³ points out, as yet unwarranted because first, our methods of studying antibodies are still too crude, and second, antibodies may actually exist in the cells while remaining undemonstrable in the serum. Moreover, isolated instances have been recorded of allergic asthma developing after the original introduction of a foreign protein. Walker⁴ has reported horse asthma persisting after the administration of horse serum and Sewall⁴ has recorded a similar case in a laboratory worker after the injection of rabbit serum.

Besides this, there has been at least one case of passive transfer of sensitization in humans by the introduction of the serum of an allergic individual into a nonallergic. This may be interpreted as indicating the presence of antibodies in the blood. Ramirez⁵ describes the case of a man who developed an attack of asthma for the first time in his life when he went driving in a horse and carriage shortly after having received a transfusion of 600 cc of blood for pernicious anemia. The patient then showed positive skin reaction to horse dander, and it was found that the donor of the blood was a horse asthmatic and was likewise sensitive to horse dander by skin test.

Indeed, Prausnitz and Kuster⁵ have developed a method of skin testing to be used, for example, in individuals with urticaria who give multiple non-specific reactions to the skin test. This method consists in procuring serum from the allergic person, administering it intradermally to a nonallergic person, and, twenty-four hours later testing the latter with the various proteins, at the exact site of passive sensitization.

Of course, not all allergic patients are born such. I have had many patients developing symptoms even past middle life who have been sensitive to such common articles as wheat, egg, feathers, and the like, and who have had contact with them sometimes for half a century or more before developing any symptoms suggestive of allergy. I have seen sensitization to one protein develop in individuals previously nonsensitive to that protein, although they were sensitive to others.

Why do some people develop hay fever and asthma while others with similar local pathology and similar protein exposures do not?

With inhalent proteins it is possible that mechanical abnormalities in the nose play a part. It has been shown that proteins, such as pollen protein, may be digested to a certain extent by the normal nasal secretion. With defective aeration, as behind a nasal spur, abnormal quantities of mucus collect, and here it is conceivable that protein is absorbed through the nasal mucosa. There is also evidence that the absorption of undigested protein through an apparently intact mucous membrane renders that surface more permeable for subsequent absorption of protein. Sewall has produced sensitization in guinea pigs by the application of horse serum to the nasal mucous membrane. Therefore, an overdose or massive dose of protein applied to the nasal mucosa might overcome the resistance of any individual irrespective of local pathology.

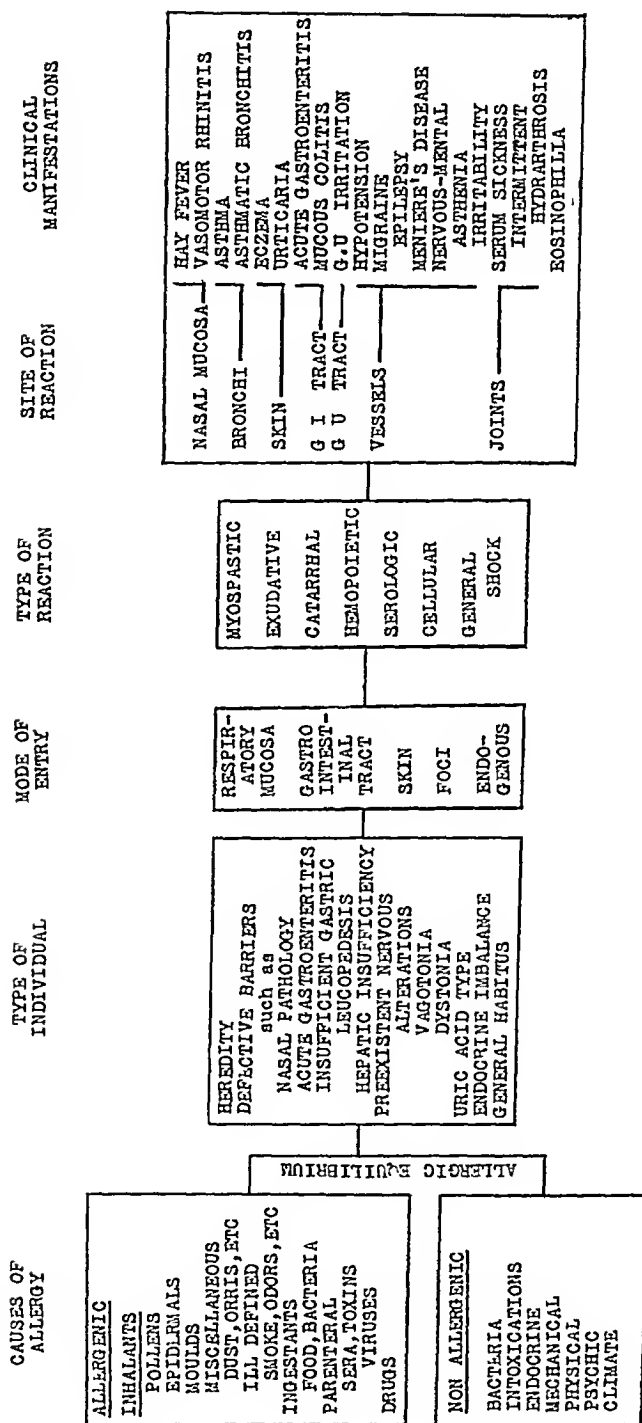
Most of the work on experimental anaphylaxis has been done on guinea pigs. It is true that there are many points of difference between human allergy and guinea pig anaphylaxis but it is equally true that there is as great a difference between anaphylaxis as observed in guinea pigs and that observed in other experimental animals such as the rabbit, dog, and horse. The chief recognizable phenomenon in experimental anaphylaxis is smooth muscle spasm. In guinea pigs the bronchial musculature is most active although as is well known, the uterine muscle also contracts. In anaphylactic rabbits there is little spasm of the bronchial muscles but pronounced spasticity of the muscles of the pulmonary arteries and arterioles. Consequently the symptoms are quite different. In dogs the smooth muscle preponderance appears to be in the portal circulation and the symptoms of anaphylaxis are gastrointestinal rather than respiratory.

The outstanding feature in dogs is a pronounced fall in blood pressure. With this, but apparently not dependent on it, there is an increased flow of lymph and a severe local reaction in the intestines. This amounts practically to an acute enteritis with edema and petichial hemorrhages in the mucous membrane of the gut, the lumen of which is often filled with mucus mixed with blood. Weil⁹ suggests that the differing symptoms in these three types of laboratory animals are due primarily to differences in quantitative distribution of smooth muscle. He finds an astonishing development of the bronchial musculature in normal guinea pigs while the pulmonary arteries of rabbits present a remarkable degree of muscular development. The walls of the hepatic veins of dogs differ from those of the other animals in showing again a high muscular development. This suggests an active speculation as to why some allergic persons develop hay fever or asthma, others headaches or the symptoms of an acute gastroenteritis while others show predominantly dermal manifestations.

I have found protein sensitization to be a factor in such apparently diversified and unrelated conditions as hay fever, vasomotor rhinitis, asthma, eczema, urticaria, acute gastroenteritis, mucous colitis, hypotension, migraine, certain mental states, serum sickness and intermittent hydrarthrosis. Other immunologists have added still more conditions to this list. It is certainly conceivable that with different types of reacting individuals with differing modes of entry of the foreign protein into the body, with different types of reactions as enumerated on the accompanying chart and with differently located preponderances of the reacting tissues within the body, the allergic explosion will manifest itself in a wide variety of clinical forms.

I have prepared the accompanying chart to correlate the many etiologic factors and clinical manifestations of allergy. Study of the chart will show very easily how many factors may be at work and how many symptoms may result even though the basic underlying conditions remain the same. The chart also illustrates diagrammatically my theory of allergic balance or equilibrium.

The immediate exciting causes of allergic reactions, whatever their type, are most varied. The direct etiologic agents are usually specifically allergenic,



that is, foreign protein. However nonspecific nonallergenic factors may also be at work. Usually both types play a part although the former plays a preponderant one.

The more we learn from our study of these various clinical conditions as allergic phenomena the greater has been the number of cases that we have been able to relieve or completely cure by specific therapy but at the same time the more closely have we been forced to a conclusion that allergy and allergic disease cannot be explained in its entirety purely on a basis of protein sensitization. We find asthmatic or eczematous individuals clearly sensitive by skin test to several different proteins some of which produce no symptoms after exposure. If an individual is sensitive to two proteins apparently in equal degree why does but one cause symptoms? How are we to account for Raekemann's observation that 10 per cent of normal healthy children who have had no evidence of allergy are sensitive to one or more foreign proteins? It is true that 13 per cent of these did develop allergic manifestations after various intervals. But why had they not developed them at the same time that they developed the capability of giving positive skin reactions, especially when they were coming in sometimes daily contact with the offending protein?

Take a group of individuals all sensitive among other things to the protein of wheat, all relieved by the elimination of wheat from the diet and all or practically all having experienced recurrence of symptoms when again eating wheat thereby proving the etiologic position of this protein. One has bronchial asthma, another recurrent urticaria, several have eczema, one involving the back of the neck, another the left hand and fingers, another the perianal region and still another with a generalized eczema involving practically the entire body. Why the difference in the localization within the body when all are sensitive to the same protein and apparently in like degree and when all are coming into regular daily contact therewith?

In the case of eczema we may explain this as due to the added action of a nonspecific factor. Thus mechanical irritation, the rubbing of the collar on the neck, moisture and friction in the perianal region or mechanical trauma of the hand. Likewise we may think of bronchial asthma as occurring because of some inherent diminished resistance of the mucosa of the respiratory tract, from preexisting nonspecific respiratory infection etc., but that this is not the entire explanation is indicated by observation of the same varieties of localization in otherwise healthy infants, none of whom have been subject to precedent disease.

Again, with an individual sensitive to some protein with which he comes in daily contact, why is it that at times he remains symptom free, the disease manifesting itself only in attacks?

Two answers present themselves to explain in part the various questions raised. First in all probability nonspecific factors do act often coincidentally with specific agents, both in the precipitation of allergic symptoms and at the same time in determining the localization thereof. Second, even this conception of an interaction between specific and nonspecific causative factors will not answer all of the questions raised because our conception of the patho-

genesis of allergy is still incomplete We now discuss it purely in terms of protein sensitization The true conception will probably not be arrived at until we have progressed to where we can discuss it in terms of intracellular activity, in terms of the chemistry or physical chemistry of the individual cells located in various regions of the body Thus, localized urticaria from heat or light or asthma from exposure to cold or wind, must ultimately be explained in terms of cellular activity

On the other hand, take the sensitive child who has never had symptoms from exposure to a protein to which he is sensitive Take the individual sensitive to a protein with which he comes in daily almost constant, contact but who manifests symptoms therefrom only at intervals I look upon these individuals during the intervals of freedom as being in what I have termed a *balanced allergic state* The patient is sensitive but by some mechanism still obscure, maintains a normal balance It is useless in the present state of our knowledge to attempt to localize the mechanism by which this is accomplished We may speak of the detoxicating action of the liver or of the activities of the endocrine system, but so far the part which they play is purely hypothetical At any rate the individual receives the allergenic protein within the system and disposes of it somehow without developing any of the various allergic manifestations, but allow some additional factor to overthrow this governing mechanism and symptoms will ensue The additional factor may be *either specific or nonspecific* It may be an overdose of the same allergen Thus, a child in my experience, sensitive to the protein of chocolate, can ingest small amounts with impunity while large amounts overthrow the balance and precipitate asthma A patient with eczema of the hands, sensitive to wheat protein noted that her symptoms cleared up on a postwar visit to Germany, where the amount of available wheat flour was distinctly limited

An individual may be sensitive to several proteins, as for example, foods, and satisfactorily metabolize one of them but develop symptoms when the mechanism becomes overwhelmed by the simultaneous ingestion of two or more

A woman with autumnal hay fever due to ragweed and without symptoms of other allergic disease, aside from recurrent urticaria, experienced but 25 per cent improvement in her hay fever following pollen desensitization She was then found sensitive to several foods, including wheat and egg, and upon eliminating these from the diet, not only was her urticaria relieved, but, which is intensely interesting, her hay fever disappeared completely

These are examples of allergic unbalance, following the action of specific etiologic agents

Again the protective mechanism may become deranged through *nonspecific* causes In eczema particularly we observe outbreaks associated with unusual constipation The effect of teething on asthma or eczema is well known Intercurrent infections and focal infections may act apparently non-specifically It is still a moot question as to whether bacterial protein may act at the same time as a *specific* allergen

Intestinal upsets of various sorts, prolonged exhaustion, emotional upheaval, may become nonspecific provocative factors A boy of thirteen with

perennial asthma relieved for a period sufficiently long to be conclusive, by the removal of the offending food proteins and removal from association with feathers, had an altercation with his friends. One boy starts throwing stones at him. The patient has to protect himself behind a telephone post. Within ten minutes he is in a violent attack of true bronchial asthma. He has eaten nothing and his environment has not changed. A child five years old, sensitive to the proteins of wheat, strawberries and chocolate who has been symptom free for two years on dietary treatment falls by accident into a swimming pool. He is rescued at once scarcely having had time to sink beneath the surface of the water. An attack of asthma follows shortly. This may have been due to fright or to sudden cold but whatever the cause, it was nonspecific in nature.

Many an individual having discovered those articles to which he is sensitive, can remain symptom free until he develops some intercurrent infection such as acute tonsillitis which appears to overthrow the allergic balance.

The effect of mechanical or chemical irritation is experimentally shown in the work of Auer.⁷ Xylol applied to the ears of *nonsensitized* rabbits produced no great degree of inflammation. If on the other hand, xylol was applied to *sensitized* animals which were then given otherwise ineffective doses of antigen local necrosis occurred. He concluded that the inflammatory action of xylol caused accumulation of effective amounts of antigen in the locality affected. The practical application of this will be seen especially in eczema.

Nonallergic pathologic conditions in the body may play a part. Nasal polyps have been recognized as etiologic agents for years. Hernia has been mentioned. Abdominal allergy is more likely to occur in individuals in whom there is some other pathology in the intestinal tract. Disease of the endocrine system plays a part the extent of which we do not yet know. Thus, the case has been reported of a woman who developed bronchial asthma shortly after the production of an artificial menopause. She was treated over a period of practically two years with the elimination of those proteins to which she had been found sensitive without benefit whatsoever. Three days after the exhibition of ovarian and mammary extract, it is reported that her asthma ceased. I have the record of a middle aged woman sensitive to several food and epidermal proteins who did not respond to removal of the allergens to autogenous vaccine to peptone treatment or to the administration of calcium chloride intravenously but who promptly recovered following the administration of thyroid and parathyroid extracts the former in relatively large doses. It is of interest to note that her attacks came usually with the catamenia.

Many causes may interact in the production of symptoms but the basis of treatment is most assuredly the sensitization test. The nose and throat man can often return a patient to allergic balance by removal of foci, polyps and the like. The dermatologist can relieve eczema by the removal of local irritation and by the application of soothing ointments. The pediatrician can do likewise by general dietary readjustments but they have many failures, and the patient, still allergic is but in a temporary state of equilibrium which may again be overthrown by any of several factors. I may say without great

Galup and Ségaud¹³ bring out that the phenomenon might equally be explained as a reflex vasomotor manifestation, resulting from contact of milk with the gastric mucosa in vagotonic individuals

Is there an allergic diathesis? Is there a nervous predisposition to such diseases as asthma or migraine? Is this a basic factor or is it coincidental? Allergy appears to occur especially in the vagotonic type of individual. Vagotonia is, however, by no means a constant finding in allergic patients. Perhaps more frequently we observe a dystonia, an imbalance between the sympathetic and parasympathetic innervation with alternating dominance of one or the other nerve groups

Some writers, particularly those in France, believe there is some association between a uric acid diathesis and the allergic state. They mention a frequent occurrence of gout with asthma and state that the onset of an asthmatic outbreak may sometimes be foretold by the disappearance of urates from the urine

There is much clinical evidence of an endocrine factor in the allergic disturbances, such as onset or termination at or near adolescence or the menopause, the relationship of attacks to the catamenia, and the result of opotherapy in selected cases

I have said that smooth muscle spasm is the outstanding recognizable experimental evidence of anaphylactic reaction. This myospastic reaction is, however, accompanied by other reactions more difficult to follow. All of these reactions probably play a part in most allergic explosions. Huber and Koessler,¹⁴ for example, have shown that the pathology of bronchial asthma consists not only of a smooth muscle hypertrophy in the bronchial tree but in a definite exudation of serum with a catarrh of the secreting epithelium. The hemopoietic reactions are eosinophilia and sometimes leucopenia. Coca¹⁵ has emphasized the importance of the state of activity of the reactive tissue, or "shock organ," at the time of contact with the allergen or atopen, no matter whether this tissue is located in the bronchi, the nasal mucosa, the skin, or elsewhere

Whether or not we grant the presence of antibodies in allergy, the blood serum plays a great part in the reaction. Call it antigen-antibody reaction, disturbance of colloidal equilibrium, or whatever the prevailing fashion of the day, the fluids of the body play some part even though they may not be the exact site of the allergic reaction. I will venture to prophesy, however, that when the last word has been said on anaphylaxis and allergy, these phenomena will be discussed in terms of the vital activity of the individual living cells

Having seen how many different types of factors may interact to produce allergic symptoms, how the functional state of the reacting body plays a part, how the direct causative agents may enter at various places in the body, and how the reaction is expressed by a variety of types of responses, one should have little difficulty in understanding how the clinical reactions may take on a wide variety of forms. On my chart I have enumerated those disease conditions which, in my own experience and in the experience of others, appear often to be influenced by the allergic state. I do not mean to say that allergy

is the cause for all of these diseases. Indeed, my entire thesis has been that it is but one of many causes, sometimes the most important, sometimes perhaps the least important, but I will say that treatment instituted on the basis of positive sensitization reactions and perhaps modified in accordance with other factors which we have discussed has often enabled me to give relief to sufferers from any of this group of diseases, where other methods have previously failed.

Not all of my cases of migraine for instance, are allergic. I have found an allergic factor in 37 per cent of migraine cases¹⁵. The remainder may or may not be allergic but with this 37 per cent I obtained relief where other treatment had failed. Fifty five per cent of my eczema series¹⁶ were relieved by protein avoidance alone. The other 45 per cent may not have been allergic but it is worthy of note that in the series that did not experience relief there were nearly as many positive skin tests as in the other series. This was also true for the personal history of allergic diseases other than the eczema and for family histories of allergic disease. It seems probable therefore that allergy was also a factor in a number of these poor result cases, but that some of the other factors which we have discussed prevented satisfactory results.

I make no pretense that the chart which I have presented is complete or inclusive. Exception might well be taken to some of my classifications, but I do feel that it shows more clearly than any prolonged discussion how clinical allergy is a disease of many ramifications, how many apparently unrelated or even contradictory observations may be correlated into the clinical picture, and how the clinical manifestations may take on any of a wide variety of forms.

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REPORT OF A CASE OF RENAL DIABETES ASSOCIATED WITH DIABETES MELLITUS*

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THE term "renal diabetes" properly refers only to those instances of glycosuria accompanied by a normal or subnormal blood-sugar level. When a discussion of this condition arises, one is already cautioned to be certain that the condition be not really diabetes mellitus, and if any disturbance of glucose utilization be present, the conclusion seems to be that a diagnosis of renal diabetes is not justified. We feel that such an attitude is not correct, and we present this case as a justification of our stand. We believe that an excretion of sugar by the kidneys of a patient with diabetes mellitus, at a time when that patient's blood-sugar level is normal or subnormal, just as truly indicates the presence of renal diabetes as would a similar excretion in a patient without diabetes mellitus.

In cases of diabetes mellitus of long standing it is usually observed that the kidney threshold for glucose rises and that diabetic patients may often have a fasting blood sugar of 200 mg per 100 cc or over, and still show no glycosuria. We have had one patient this year whose fasting blood sugar rose to 250 mg without the presence of glycosuria. We make these statements to emphasize further the importance of diagnosing renal diabetes in the presence of diabetes mellitus when sugar excretion occurs at a normal or low blood-sugar level. The bearing of such a diagnosis on the diabetic treatment is evident, since the urinary sugar is usually accepted as the most convenient guide. We shall discuss these points after presenting the details of the case.

L T Y (P U M C Hospital No 14607), a Chinese man, aged fifty six, had had polydipsia and polyuria for twenty five years, although otherwise in good health. A urinary examination in June, 1926, disclosed the presence of sugar, but no other symptom of diabetes was present. He was admitted to the P U M C hospital on August 24, 1926. Physical examination was negative. The blood pressure was normal. Renal function test showed a phthalein excretion of 67 per cent in two hours. Blood and urine examinations were negative, except for glucose abnormalities. At this time his fasting blood sugar was 200 mg (Folin and Wu method) and the urinary output (without dietary control) was in the neighborhood of 80 gm in twenty four hours. After nine days on a 2000 calorie diet with a fatty and glucose ratio of 1.5, and thirteen days on 1880 calories with a ratio of 1.6, the glucose excretion gradually fell to between 6 and 8 grams per day, accompanied by a loss of body weight from 90 to 85.6 kilos. At the end of this period his fasting blood sugar was 71 mg, although at all periods of the day glycosuria was present. Glucose tolerance test, performed at this time, gave the following results:

	<i>Fasting</i>	<i>After ½ hour</i>	<i>1½ hours</i>	<i>3 hours</i>
Blood sugar in mg	71	166	181	133
Urinary sugar per cent	0.9	2.7	8.3	5.5

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Administration of from 6 to 7 units of insulin daily made no difference in the sugar excretion. He left the hospital against advice and we later learned that the physician in charge of his treatment could get no satisfactory effect of insulin using even up to 40 units daily. He was readmitted for study November 29, 1926, with a blood sugar of 133 mg and a sugar output in the urine of 27 grams in twenty-four hours. In the course of five days on a 2000 calorie diet (ratio 2.0) the sugar output fell to 7 grams per day and on 2140 calories (ratio of 2.47) the output was maintained between 4.4 and 7.3 grams for two weeks while the body weight fell from 87.4 to 86.1 kilos. The administration of 15 units of insulin daily for five days gave a very slight effect keeping the output between 3.9 and 4.9 grams per day. Fasting blood sugar after one week of this diet and before giving the 15 units of insulin daily was 66.6 mg.

A sugar tolerance test performed at the end of this dietary period using 0.2 cc of blood from finger puncture for each determination gave

	Fasting	After $\frac{1}{2}$ hour	1 $\frac{1}{2}$ hours	3 hours
Blood sugar in mg	135	309	250	153
Urinary sugar per cent	0.45	3.8	5.0	4.1

The patient was discharged from the hospital with advice to his physician to allow him an adequate diet and to use insulin sufficient to keep the urinary output approximately 8 grams a day. With the diagnosis of renal diabetes associated with the diabetes mellitus and the knowledge of the amount of urinary sugar to be accounted for on that basis the treatment now became satisfactory to both physician and patient.

DISCUSSION OF THE CASE

We had here a patient with definite findings of diabetes mellitus as evidenced by the blood sugar curve in the glucose tolerance test and by the marked lessening of urinary sugar on reduction of glucose intake or with insulin administration. However on a low food intake with or without insulin, the sugar excretion continued at times when the blood sugar level was normal or below normal (71 mg, 66.6 mg, 111 mg). On the basis of these findings we made a definite diagnosis of renal diabetes associated with diabetes mellitus and advised that in the treatment of the latter disease the urinary sugar accountable for by the former should be disregarded. The lack of any evidence of nephritic changes other than the reduced glucose threshold places this case with the less frequently found type of renal diabetes which is not associated with chronic nephritis.

A SPECIES OF *ESCHERICHIA* FROM GASEOUS INFECTION*

By FREDERICK W SHAW, M D, RICHMOND, VA

WEINBERG and Seguin,¹ from a study of 126 cases of gaseous wound infection other than tetanus, found six cases of the phlegmonous type which yielded aerobes only. It is thus concluded that aerobes are apparently able to produce gaseous phlegmons, but the number of such cases is small. The organism to be described in this paper was isolated in pure culture from the deep structures of the lesion. It produced a gaseous infection in rabbits, from the lesions of which it was recovered in pure culture.

CASE REPORT

R. G., white male, entered the Hospital Division, Medical College of Virginia. The left patella was fractured, and there were numerous abrasions over the lower and upper extremities. There was a laceration of the inner aspect of the left lower thigh. Four days later the patient was delirious, his temperature was 103° F and his pulse 130. Examination of the laceration at the inner side of the left knee showed an inflamed area of about 6 cm. Crepitation was present and extended to the groin. The left leg was amputated at the hip.

A gross description of the leg showed a wound 6 cm in length penetrating the skin on the lateral aspect of the knee. The subcutaneous tissue of the anterior and lateral aspect of the leg were crepitant. On section the subcutaneous tissue and the intermuscular fasciæ of the region were filled with an extensive hemorrhagic edema. There was definite involvement of the superficial muscles and at approximately the same line some of the deep muscles were more involved and appeared dark and soft. There was no odor.

A variety of culture media were inoculated from the deep structures. Gas was given off from the anaerobic meat media in six hours. All of the culture media showed a Gram negative, nonspore forming rod. No other organism was observed. Subcutaneous inoculations into guinea pigs killed them in twenty-four hours. Autopsy showed a septicæmia. Subcutaneous inoculations into rabbits showed a definite area of beginning crepitation at the area of inoculation, beginning in about forty-eight hours. On the sixth day the lesions showed a crepitant area of about 5 cm. Incision of this area revealed an inflammation of the underlying muscles accompanied by the presence of gas. There was no odor. The original microorganism was isolated in pure culture from the heart's blood of the guinea pigs and from the lesions in the rabbits.

BACTERIOLOGY

A description of the organism follows.

Short rods, motile, Gram-negative, no spores.

Aerobic and facultative anaerobic.

Gelatin not liquefied.

Agar colonies whitish-gray, slightly spreading.

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†The patient's temperature reached normal in six weeks and he was discharged two weeks later.

Broth turbid with sediment No pellicle

Serum not liquefied

Milk acid and coagulated

Indol is formed

Nitrites reduced

Lead acetate not blackened

Acetyl-methyl-carbinol not formed

Acid and gas in lactose, mannitol, dextrose, maltose, arabinose, galactose, levulose, rhamnose, glycerol, xylose, and trehalose

No acid or gas in saccharose, dulcitol, dextrin, raffinose, adonitol, mulin, sorbitol, inositol, salicin or amygdalin

This microorganism is readily differentiated from *Escherichia coli* by its inability to ferment dulcitol. It closely resembles *Escherichia giunthali* (Morgan) Castellani and Chalmers, and *Escherichia paragiunthali*, Castellani and Chalmers (Table I), but it may be differentiated from these by its inability to produce gas in dextrin, raffinose and sorbitol.

The name *Escherichia emphysemata* is suggested.

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LABORATORY METHODS

A MODIFICATION OF THE McLEAN VAN SLYKE METHOD FOR THE ESTIMATION OF CHLORIDES IN BLOOD*

BY M I HANNA B A TORONTO CANADA

IN 1915 McLean and Van Slyke¹ introduced an iodometric technic for the determination of chlorides in small amounts of body fluids. Van Slyke and Donleavy² and Austin and Van Slyke³ simplified the earlier method of protein precipitations so that accurate determinations of chlorides in plasma and whole blood could be made. Gettler⁴ and later Short and Gellis⁵ have modified this method in order to make it applicable to blood filtrates made by the Folin Wu tungstic acid method. Though all of these methods are sufficiently accurate and satisfactory for estimation of chlorides in the laboratory, the technical procedures require too much time and too much material to be entirely suitable for clinical work. The following modification of the McLean Van Slyke method has been found to fit well into the Folin Wu system of blood analysis without an undue waste of time which may be an important feature, particularly when dealing with cases of intestinal paresis. Further, the same blood filtrate which is required for other determinations on the patient may be used for estimating the chloride content of the blood.

METHOD

One part of oxalated blood or plasma is diluted with seven parts of water, and the pipette is washed with the diluted blood and drained twice. One part of 10 per cent sodium tungstate and one part of $2/3$ N H₂SO₄ is then added as in the original Folin Wu method. The mixture is vigorously shaken for a few minutes and filtered through a No. 40 Whatman filter paper into a dry flask. To 10 c.c. of filtrate are added 10 c.c. of distilled water and 5 c.c. of the M/29.25 silver nitrate solution (see Solutions). Allow to stand five minutes, then filter once through two small No. 40 Whatman filter papers folded together. A few drops are poured down the sextupled side of the filter and allowed to soak into the paper. The remainder is then poured into the filter. To 10 c.c. of filtrate, add 2 c.c. of starch citrate nitrate solution and titrate to the first permanent blue tint with M/73.1 potassium iodide solution, using a 5 c.c. standardized burette with a capillary tip dropping from 50 to 60 drops per c.c.

10.15 = double the titration value = mg NaCl per c.c. of original blood

EXPERIMENTAL

Several standard solutions of hydrochloric acid were made up by Hulett and Bonner's method and checked against alkali. They agreed satisfactorily.

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Samples of standard HCl were then analyzed for chloride content by the Austin-Van Slyke method and by the above modification. The results are shown in Table I.

In Table II are recorded a series of determinations on whole blood by the Austin-Van Slyke method and by the proposed modification. The maximum difference in titration values in the two methods is equivalent to 10 mg of NaCl per 100 cc of blood, the maximal difference between all duplicates is equivalent to 6 mg per 100 cc of blood.

TABLE I
CHLORIDE AS NaCl IN STANDARD HCL SOLUTIONS
(Theoretical Content 584.6 Mg Per 100 CC)

	AUSTIN VAN SLYKE METHOD	MODIFIED METHOD
	found	found
1	590	580
2	592	582

TABLE II
THE CHLORIDE CONTENT OF BLOOD BY THE AUSTIN VAN SLYKE METHOD AND BY
THE PROPOSED MODIFICATION
(As Mg NaCl Per 100 CC of Blood)

SPECIMEN	AUSTIN VAN SLYKE METHOD	PROPOSED METHOD	PERCENTAGE DIFFERENCE
1	510 513	505 505	-1.3
2	515 518	510 511	-1.2
3	495 495	489 495	-0.6
4	485 485	489 485	+0.4
5	485 490	495 495	+1.6
6	515 515	515 519	+0.4
7	495 495	495 495	0
8	490 495	499 499	+1.4
9	515 515	519 515	+0.4
10	535 535	535 539	+0.4
11	525 525	525 525	0
12	515 512	515 515	+0.3

DISCUSSION

The modified method gives results which are shown to be comparable with those obtained with the Austin Van Slyke method. Satisfactory results are also obtained with the method in estimating chloride of plasma, pleural and ascitic fluids, urine, cerebrospinal fluid, and gastric juice, employing appropriate dilutions where necessary. With urine containing no albumin the preliminary precipitation with tungstic acid is omitted. The principal value of the method, however, lies in the small amount of blood filtrate required, the small number of operations and the speed with which the determination can be performed. If blood filtrate is limited in amount a single determination may be made using half the quantities recommended here, and it is even possible with care to obtain satisfactory results on much smaller quantities of blood though the additional trouble required will seldom make this a desirable procedure. Particular attention should be paid to four points. The reagents used must be chloride free and the glassware carefully recalibrated. After addition of the silver nitrate to the diluted blood filtrate the mixture must stand five minutes or more to permit of diffusion of occluded silver nitrate, and lastly, the double filter paper used should be Whatman No. 40. Doubtless other brands and qualities of filter paper may be found satisfactory, but thus far among those tested this particular kind has been the only one which combined rapid filtration with complete retention of the precipitated silver chloride on the first filtration. As has previously been pointed out by others in connection with other determinations on blood filtrates the use of an excessive quantity of oxalate as an anticoagulant is to be avoided.

SUMMARY

A modification of the methods devised by Van Slyke and his collaborators for the estimation of chloride in blood, which is applicable to Folin Wu tungstic acid filtrates, is here described. The method is shown to be accurate within 1 per cent or of the same order of accuracy as the original method. The advantage of the modification lies in the smaller amount of time of work and of blood filtrate required for the determination.

SOLUTIONS

M/29.25 Silver Nitrate Solution (1)

Pure fused AgNO_3	5.812 gm
HNO_3 , Sp. C. 1.42	250 cc
Water to	1000 cc.

M/75.1 Potassium Iodide Solution (2)

Pure KI	2.27 gm per liter
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Two and four tenths gm KI are diluted to a liter and used to titrate 5 cc of the silver nitrate solution with 5 cc of starch citrate nitrate solution and 5 cc of water. The strength of the solution is then adjusted so that exactly 12.65 cc of the KI solution is required (0.15 cc is used for obtaining the end point 12.50 cc to precipitate the silver).

Starch Citrate Nitrate Solution (1)

Sodium citrate $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ $5\frac{1}{2}$ H_2O	446 gm
Sodium nitrite	20 gm
Soluble starch	25 gm

Dissolve the starch in 500 cc of water by boiling for several minutes add the citrate and nitrate and heat until all is dissolved Filter through cotton, wash the filter with hot water, and when cold, make up to 1000 cc

This solution is satisfactory for at least six weeks When it is required infrequently, Short and Gellis' solution has some advantages The same amounts of citrate and nitrate are dissolved in 800 cc of water and made up to 1,350 cc The starch is made up weekly by boiling in 100 cc of water and diluting when cold to 150 cc The two solutions are mixed before use in the proportion of 1 to 9 Three cc of the mixture replaces 2 cc of the McLean Van Slyke starch citrate nitrate solution

I wish to thank Dr W R Campbell, who suggested this work, for his constant interest and help

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A SIMPLIFIED TECHNIC FOR STUDYING THE SECRETION OF LYMPH*

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THE method in general use for studying the formation and flow of lymph in class or laboratory courses in physiology consists in collecting the lymph by means of a cannula placed in the thoracic duct A meal containing large amounts of fat given several hours before the experiment greatly facilitates finding the duct, but even under these favorable conditions the operator often experiences considerable difficulty in differentiating the duct from strands of fat in the neighborhood, especially if the field is to any extent obscured by hemorrhage This is entirely obviated, and the duct can be made to stand out conspicuously if to the meal previously given is added a small amount of fat (we have used butter) which has been saturated with some fat dye Sudan III answers the purpose admirably This method may also be of advantage in studying the course of the intestinal lymphatics, as after feeding the dye they stand out in marked contrast to the strands of fat in the mesentery and along the blood vessels

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A SIMPLE ARTIFICIAL RESPIRATION APPARATUS*

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AN APPARATUS for the administration of artificial respiration was devised by Dr. A. L. Tatum by using an air driven windshield wiper of automobiles. The windshield wiper which was used is manufactured and patented by Folberth, Cleveland, Ohio. Dr. Tatum did not publish a description of his apparatus. It is a simplified adaptation of the Becker apparatus.

This instrument, however, was not very adjustable as the outflow of air which corresponds to the inspiratory phase of respiration was considerably longer than the phase corresponding to the expiration. Furthermore, there was practically no pause between inspiration and expiration so that the lungs did not have an opportunity to collapse thoroughly. We have made an attempt to overcome some of these difficulties.

The windshield wiper proper will not be described in this paper because it may readily be purchased on the open market. As far as it is known, it operates on the same principle as the ordinary positive pressure air driven windshield wiper. Only those parts which are essential for its modification for the use of administration of artificial respiration will be described.

The only changes made in the mechanism were: first, a metal shaft *S* (Fig. 1) which operates the valve *E* was made to replace the original one. This shaft was made $3\frac{1}{8}$ inches in length and $\frac{1}{8}$ of an inch in diameter. It is attached to the stopcock of valve *E* as shown in Fig. 1 and in the photograph (Fig. 5). It is so adjusted on the rock pin inside the cylinder of the air driven apparatus that it is allowed to rotate only 135 degrees—this is important in regard to the regulation of the outflow of air corresponding to inspiration. Incidentally this adjustment is very easily made. The only other change made in the air driven mechanism is that an ordinary needle valve (*V*₁, Fig. 1) is inserted in an aperture, already present in the apparatus.

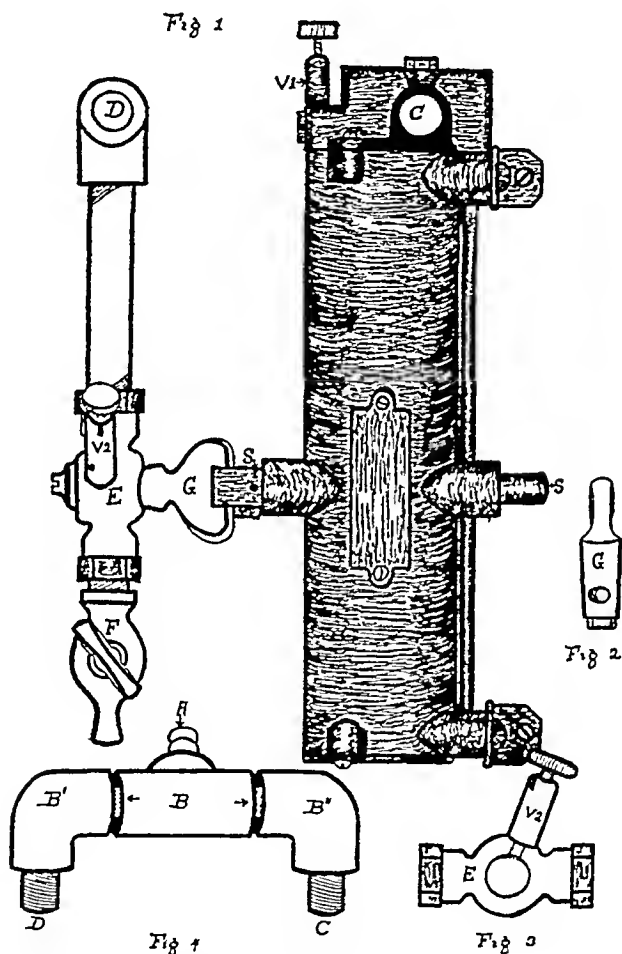
Fig. 1 shows the arrangement necessary for the conversion of this type of instrument for artificial respiration. Fig. 4 in the diagram is the connecting unit which joins the cylinder with the other portion of the apparatus, that is, *C'* is inserted at the point *C* into the cylinder and *D* is inserted into *D*. Dr. Tatum found that the diameter of the metal tubes *A* and *B*, was important as it is essential to distribute the proper amount of air to the cylinder of the air driven apparatus and also to allow enough air for the animal. The diameter of this tubing is $\frac{3}{4}$ of an inch.

Compressed air from the pipe line in the laboratory enters at *A* (Fig. 4) and goes into pipe *B*, here a part of the air goes to *B* and a part to *B'*. The

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air which goes in the former direction is used by the animal, the air going in the latter direction is used to operate the air driven mechanism. As explained above, the mechanism rotates the shaft *S* (Fig 1) which in turn operates the valve *E*, which is merely a petcock valve. This valve, however, has a modification. The hole passing through the stopcock *G* is enlarged as shown in Fig 2 by the shaded portion. Only the upper side is made larger, the hole in the lower part is not changed.



Figs 1 to 4—Diagram of artificial respiration apparatus (Reduced one half)

Valve *E* has one more modification as shown in Fig 3. A hole is drilled a bit off center on the upper surface of the petcock valve *E*, so as to coincide with the elliptical half of the hole made in the stopcock *G* of this valve. A needle valve (or another petcock valve) is placed over this drilled hole as shown in the diagram. By changing the size of this aperture, the ratio of inspiration to expiration may be altered. It was found that when the needle-valve *V*₂ is closed, the ratio of inspiration to expiration is 1 to 1, when it is widely opened, the ratio is 1 to 5. By appropriate adjustment of the valve, the intermediate ratios may be obtained. Incidentally, this arrangement also makes it possible

to operate the mechanism with less air, so that it may be used for very small animals or in cases where only a small amount of air is desired

The volume of air delivered at the outlet is controlled by the stopcock valve *F* (Fig 1) the volume may also be varied by the amount of air allowed to enter at *A* (Fig 4) The rapidity which the stopcock *G* of valve *E* (Fig 1) is operated is controlled by the needle valve *V*₁ on the cylinder, which has been described above It may also be said that the rapidity with which this stopcock is turned in a given time is dependent to some extent on the air pressure



Fig 5—Artificial respiration apparatus

entering at *A*, but this can be compensated for by resetting the valve *V*₁ The relation of inspiration to expiration is controlled by the needle valve *V*₂ as previously described In order to allow the lungs to collapse fully it is well to insert a T tube between the tracheal cannula and the outlet valve *F*

To summarize An apparatus is described in which the rate of respiration may be controlled, the volume of air at each inspiration may be controlled, and the ratio of inspiration to expiration may be controlled It was found that this instrument is admirably adapted for small animals, but it may even be used for large dogs weighing from 20 to 25 kilos This simple air interrupter moreover, may be used for purposes other than the administration of artificial respiration It is not complicated so that it does not easily get out of order and lastly, it is quite inexpensive

SIMPLIFIED APPARATUS AND TECHNIC FOR THE DETERMINATION OF THE ICTERUS INDEX*

BY RUSSELL C. PIGFORD, M.D., NEW ORLEANS

THE Meulengracht icterus index has proved a valuable adjunct in diagnostic and prognostic procedures. The technic, while comparatively simple to the average laboratory worker, offers disadvantages which tend to discourage the procedure as a routine laboratory test. The chief objections are (1) many practitioners are not equipped with the Duboseq colorimeter, (2) in some instances accurate weighing of the small quantity of the potassium dichromate for the standard cannot be accomplished, (3) at least 5 cc. of serum are required for the Duboseq apparatus, and (4) in sera with a high index repeated dilutions are necessary for the readings.

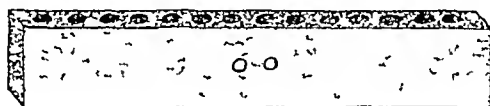


Fig. 1—Front view showing small holes

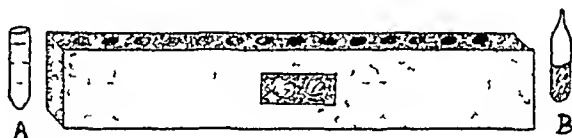


Fig. 2—Back view showing large holes and ground glass back. A, tube for unknown; B, standard tube

In the modified technic, standards of potassium dichromate solution of varying strengths are prepared and sealed in small test tubes. The strengths vary from 1/100, with an index value of 100 to 1/10,000, with a value of 1. The serial dilutions are readily made from a 1 per cent solution, acidified with a few drops of concentrated sulphuric acid. These standards are used for matching against the unknown serum in a manner similar to the technic of the phenolsulphonphthalein test.

Apparatus—A wooden block $\frac{3}{4}$ of an inch wide, 2 inches high, and 12 inches long is required. With a $\frac{7}{16}$ -inch bit 14 holes, equally spaced, are bored to a depth of $1\frac{1}{2}$ inches. From the side two holes are bored opposite holes numbers 7 and 8 (the middle holes) and communicating with these holes. Care must be exercised in boring the side holes as they are not to pass all the way through, but merely far enough to communicate with the holes from above. A piece of ground glass is placed across the two side holes. This is to act as a background for the matching. From the opposite side of the block two small holes ($\frac{1}{4}$ -inch bit) are bored so that they communicate with holes numbers 7 and 8 from above. By using the smaller holes in front, a greater con-

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trast is to be had in making the readings. The two central holes thus made act as a comparison box for the readings. One of the remaining 12 holes is to contain the empty test tube for the unknown serum. The other 11 are to hold the various graduated tubes of standards.

Twelve test tubes 9 mm (inside diameter) by 45 mm 2 cc capacity are used as containers. One is kept for the unknown. The other 11 are to contain the various strengths of the dichromate solution. After placing the solutions in the tubes they are labeled and are sealed with melted paraffin, sealing wax, or by heat.

The dilutions found most satisfactory, with their index values are given. They are

<i>Dilution</i>	<i>Index Value</i>
1 10,000	1
1 5,000	2
1 3,500	3
1 2,000	5
1 1,500	6.7
1 1,000	10
1 666	15
1 500	20
1 400	25
1 200	50
1 100	100

To make the reading 3 to 5 cc of blood is collected by venepuncture allowed to clot, and after thorough centrifugalization, 20 drops of serum are placed in the empty test tube. Oxalated plasma may be used in place of the serum. This is then matched in the box against the various standards. If the index is greater than 100, proper dilutions can readily be made by adding an equal or double quantity of water and multiplying the reading by 2 or 3. Clear serum is to be desired although slight clouding as occasionally encountered in sera collected after a heavy meal will not interfere with the reading. It must be remembered that very slight hemolysis will invalidate the reading.

The normal range of the icterus index is from 3 to 6. Latent jaundice ranges from 6 to 15. Above 15 the icterus is visible in the skin and mucous membranes. Values below 3 are encountered in the secondary anemias, while pernicious anemia will fall in the range of latent jaundice. The icterus index is of value in cases of obstructive jaundice. In these cases the relief of the obstruction will be demonstrable by means of this test before other signs are present. A series of indices in cases of doubtful malignancy will sometimes give valuable diagnostic information. In the cases of malignancy the index will remain constantly above 100 while the benign liver will show a much greater fluctuation in the values obtained.

The method here described has the following advantages

1. The equipment is inexpensive and can be made in the laboratory.
2. Determinations are rapidly made.
3. Only 1 to 15 cc of serum is required.
4. Calculations for dilutions are seldom necessary.
5. The readings obtained are sufficiently accurate for practical purposes.

A NEW ANIMAL BOARD*

BY I FOREST HUDDLESON D V M, AND E R CARLSON, D V M

IN WORKING with laboratory animals, a prerequisite is an efficient means of restraint. We have constructed an animal board which is serving as a means for restraining animals much more conveniently than any other which we have used. It is constructed in such a manner that it may be used for holding rabbits, guinea pigs, or rats.

A detailed description and photographs of the board are presented in the hope that it will appeal to others.

The board proper (Fig 1) is made of oak 1 inch in thickness, 11 inches wide, and 34 inches long. It is mounted on four legs 3 inches high. A

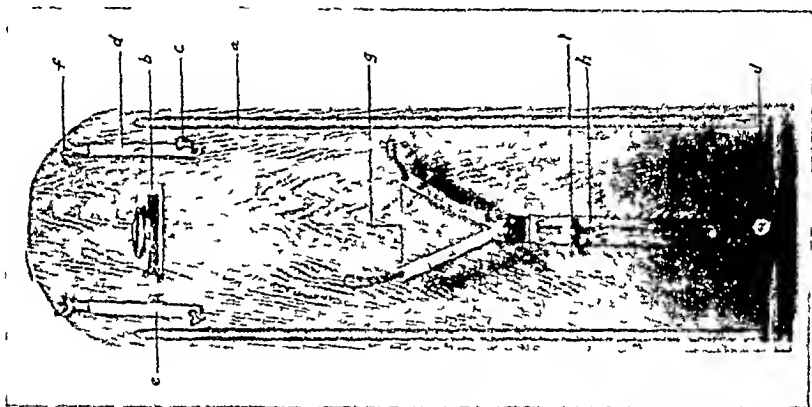


Fig 1

shallow groove (a) is cut near the edge of the top surface for collecting the excreta or blood which escapes during an operation.

Near the center of the top of the board (b) is located a half circle metal clamp for holding the head and neck. The ends of the open ring of the clamp pass downward into openings on either side of the fixed piece. The extended ends of the ring-piece are held in position by means of a set-screw situated on one side of the fixed piece of the clamp.

The anterior extremities are held in position by means of slip loops (c) made of stout cord attached to the ends of the metal arms (d). The size of the loop is determined by the position of the set-screw piece (e) which slides back and forth on the metal arm. The metal arm, which may be moved outward or inward is operated on the ratchet set-screw (f) principle. The pos

*From the Michigan Agricultural Experiment Station East Lansing Michigan
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terior extremities are held in position by loops and metal arms similar to the one just described (See Fig 2) The distance between the end of the arms is controlled from a central point (g) by a set screw ratchet The distance between the two sets of arms is controlled by the extension piece (h) which is

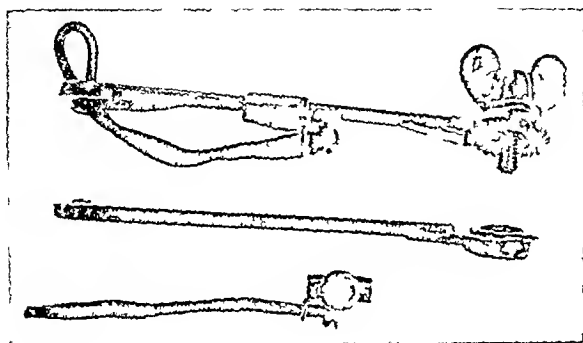


Fig. 2

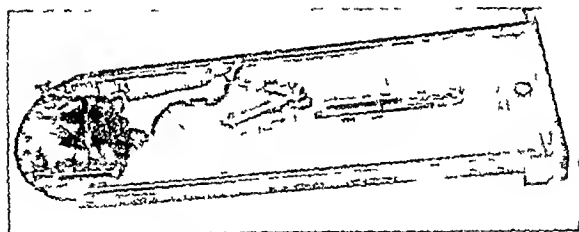


Fig. 3

held in position by a set screw (i) If one desires to make a further extension the set screw and extension may be moved back to (j) or the ends of the extension may be reversed without moving the set screw from the position shown in the figure

THE ESTIMATION OF RED CELL FRAGILITY AN HEMATOCRIT METHOD*

BY HERBERT SILVETTE, RICHMOND, VIRGINIA

VARIOUS substances have been employed to determine the resistance or the fragility of red blood cells. Of these, hypotonic solutions in varying strengths have been used with more or less satisfaction. The methods in use are those of Smith and Brown,¹ Karsner and Pearce,² Hill,³ and Giffin and Sanford,⁴ or some slight modification of these, such as that of Fontaine.⁵ They are similar in that all use hypotonic solutions of sodium chloride as a means of estimating corpuscle resistance. Simmel⁹ employs a solution which is isotonic with blood, has the same P_H value, and the same percentage of the various inorganic salts. This solution, when used in four hypotonic dilutions, is the basis of a quantitative fragility test.

It has also been shown that red cells have a constant figure of resistance towards saponin and sapotoxin hemolysis. Saponin has been used by McNeil,⁶ and by Bigland⁷ as a means of determining red cell resistance, while sapotoxin was employed by Neilson and Wheelon⁸ for the same purpose.

The hematocrit may be used to determine the fragility of red blood cells. Van Allen,¹⁰ while working out experimentally the underlying principles governing the hematocrit, pointed out that "with a decrease of the concentration of the surrounding fluid, the erythrocytes increase in size until the limit of distensibility is reached, when the volume of the cells drop almost immediately to zero." With the use of several hematocrit pipettes and a series of hypotonic solutions of sodium oxalate of different concentrations, a method for the estimation of red cell fragility becomes both feasible and practicable. Such a method was suggested by Hamburger¹² in 1912, but there is no evidence of its present use.

For rabbit's blood it has been found¹⁰ that the limit of distensibility of the red blood cells is indicated quite sharply at 0.72 per cent sodium oxalate. For human blood, the greatest volume of cells, that is, the limit of distensibility, occurs when a 0.6 per cent sodium oxalate solution is used. Invariably there is hemolysis, complete or incomplete, when a 0.5 per cent solution is employed.

It was decided, therefore, to limit the hypotonic solutions in the test to four: 0.5, 0.6, 0.7, and 0.8 per cent. The 0.5 solution would allow for any probable increase in the resistance of an unknown blood sample, while the range of 0.7 to 0.8 per cent would take care of any probable change in the direction of increased fragility.

These four hypotonic solutions should be made up as accurately as possible. To this end, about five hundred cubic centimeters of each strength

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should be prepared at a time, anhydrous sodium oxalate being used, weighed out on an analytical balance and dissolved in distilled water in volumetric flasks. The solutions keep well when the containers are tightly corked, but in order to prevent the growth of molds, a few cubic centimeters of toluene should be added to each bottle. In view of the fact that a large quantity of each strength is made up at a time, and the concentration of sodium oxalate in the various solutions differs by a relatively large degree it should be easy to secure constant results even when solutions made up at different times are used.

Four hematocrit pipettes^{10, 11} are used together with an equal number of appropriate sealing devices. Rubber bands may be used with perfect satisfaction, or the spring sealing device of van Allen¹². An efficient and inexpensive sealing band, especially suitable to this method, is shown in Fig. 1. It consists of a cork attached to a rubber band by means of a small wire staple or pin. There is a hole half way through the cork into which the



Fig. 1.—An efficient sealing band for hematocrit pipettes

upper extremity of the pipette is fitted. Also the coil is marked with the strength of the diluting fluid with which it is to be used. It is very important to mark each pipette or sealing band with the concentration of the diluent in order to minimize the chance of error due to confusing the filled pipettes, and also to facilitate reading and recording the results.

With the four diluting fluids the pipettes and the sealing bands ready the test is begun. A rather deep puncture is made in the patient's finger or ear, and the first drop of blood appearing is discarded. As a fresh quantity appears, it is drawn up into the bore of the hematocrit until the blood column is even with the top of the scale. Blood adhering to the outside of the pipette is wiped away and a quantity of the 0.5 per cent diluting fluid is drawn up until the chamber of the hematocrit is about half filled. The sealing band is now attached, first to the graduated end of the pipette, and then to the bulb end. The three other tubes are now filled and sealed in the same manner using the more concentrated solutions of sodium oxalate in turn. After a little practice it becomes easy to fill the four tubes from a

TABLE I
RESULTS OF FRAGILITY TESTS ON 50 NORMAL SUBJECTS

MALE					FEMALE				
HEMATOCRIT READINGS					HEMATOCRIT READINGS				
PER CENT SODIUM OXALATE					PER CENT SODIUM OXALATE				
	0.5	0.6	0.7	0.8		0.5	0.6	0.7	0.8
S	72	83	67	62	R	56	64	57	50
O	57	73	63	53	S	42	54	54	47
C	54	70	58	55	S	44	66	52	50
C	72	67	58	50*	R	44	64	50	49
W	49	70	52	52	W	50	56	54	43
F	54	61	55	55	G	42	54	50	45
H	45	75	59	49	F	56	64	57	45
W	33	61	56	52	E	57	61	58	52
S	45	71	61	55	W	60	63	54	49
E	56	62	49	48	T	50	57	51	48
C	61	72	59	49	V	56	65	55	46
B	74	80	63	63	G	62	65	51	45
C	47	74	61	54	F	44	66	56	53
F	51	68	53	50	A	41	58	51	45
F	61	71	50	48	C	63	55	44	44*
G	41	65	50	49	M	50	56	51	47
D	55	70	59	51	P	41	64	56	45
H	63	81	70	60	P	22	66	55	45
M	47	66	44	44	H	12	57	43	41
J	53	79	68	56	B	23	63	58	55
S	41	64	60	51	M	24	59	51	45
T	59	80	61	59	W	6	63	57	49
L	60	69	62	56	D	32	46	40	37
R	46	59	50	43	K	23	58	54	46
F	52	77	55	54	K	4	71	58	44
Av	54	76	58	53	Av	40	60	53	47

*Atypical normal curves showing increased resistance

single puncture. A little pressure some distance away from the stab is permissible, indeed necessary, but should the blood coagulate because of too slow collecting and handling, another prick must be made.

The hematocrit pipettes are now placed in the centrifuge, the even number of tubes doing away with any special counterbalancing. Centrifugalization is carried out at 2700 revolutions per minute for fifteen minutes, these having been found by van Allen to be the optimum speed and duration. It is imperative that all pipettes in a single test be centrifuged for the same length of time and at the same rate of speed in order to obtain comparative readings. After removing the pipettes from the centrifuge, the results are read and recorded.

Results are expressed and interpreted by means of a fragility curve, drawn by using the hematocrit readings as ordinates and the concentration of sodium oxalate as abscissas.

In a series of fifty healthy, normal subjects, both male and female (Table I), the greatest cell volume in each case occurred when 0.6 per cent sodium oxalate was used as the diluent. Also, in every instance some hemolysis took place using the 0.5 per cent solution, thus giving a well-defined peak for the normal fragility curve at 0.6 per cent concentration of sodium oxalate (Fig. 2). Where the peak of the curve is found to be at 0.5, that is, the greatest volume of cells obtained is with the 0.5 per cent solution, increased corpuscle resistance is indicated and the type of curve may be referred to as a

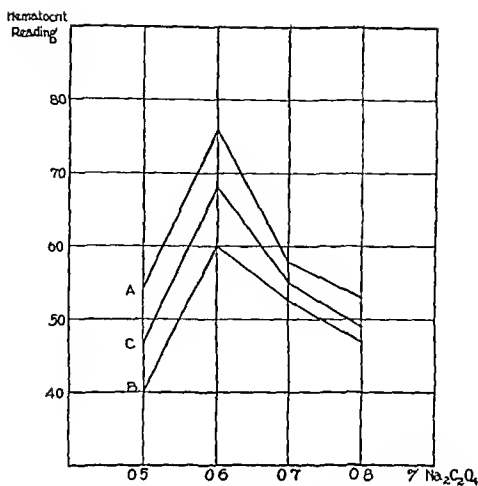


Fig 2—Average normal fragility curves. *A* average of the fragility curves of 5 normal men. *B* average of the fragility curves of 5 normal women. *C* normal fragility curve average of both sexes.

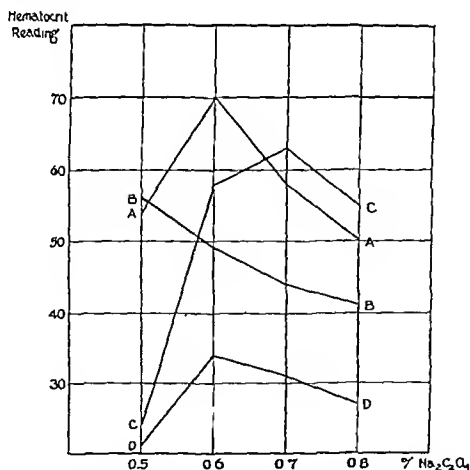


Fig 3—Typical fragility curves. *A* normal curve for comparison. *B* curve of increased resistance from a case of obstructive jaundice. *C* curve of increased fragility from a case of childhood nonobstructive jaundice. *D* normal curve from a case of severe secondary anemia.

"curve of increased resistance" (Fig 3-B) Where the peak of the curve is to the right of 0.6, either at 0.7 or at 0.8, the result may be called a "curve of increased fragility" (Fig 3-C) The degree of either resistance or fragility of the red cells is directly proportional to the degree of displacement, either to the left or to the right, of the peak of the curve in question compared with that of the normal curve at 0.6 per cent

It is also possible, by means of the angle of the line forming the left side of the peak of the curve, to interpret the results to a much finer degree than the placement of the peaks would indicate To illustrate, Fig 4-A is a normal curve, and while curves B and C in the same figure are also to be considered normal, it is noticeable that B shows a tendency toward increased

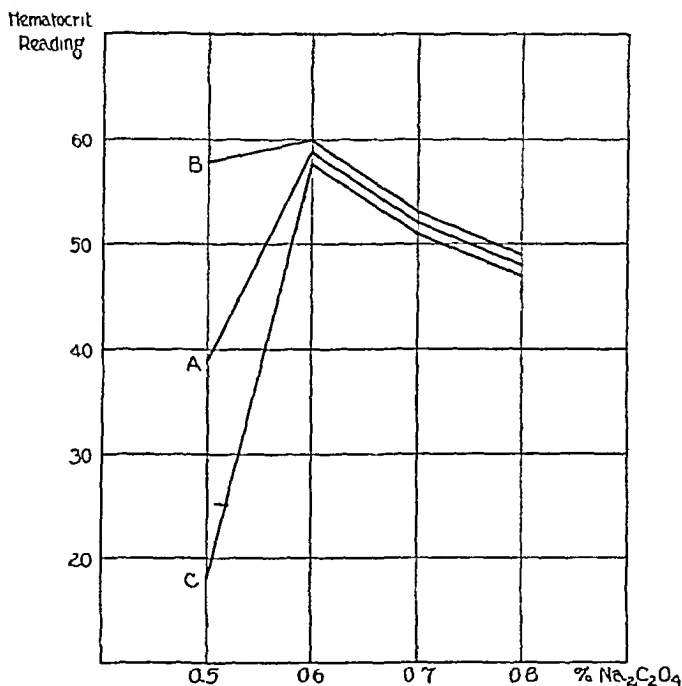


Fig 4—Normal fragility curves A average normal curve B normal curve with a tendency toward increased corpuscle resistance C, normal curve with tendency toward increased corpuscle fragility

cell resistance, and C toward increased corpuscle fragility Pathologic curves may be read to the same degree as normal ones, and in general, it will be noted that the more nearly vertical the line forming the left side of the peak, the more fragile are the cells Conversely, the more nearly horizontal that line is, the more resistant the cells Since the peak of the curve of increased resistance is not complete on the left side, this statement will not apply In that case, a falling line between 0.5 and 0.6 would denote a greater degree of corpuscle resistance and a line approaching the horizontal a somewhat lesser degree It is then possible to interpret the results of a hematocrit fragility test with as much, if not greater, accuracy as the results indicated by the two figure index of initial and complete hemolysis

TABLE II
RESULTS OF FRAGILITY TESTS ON A SERIES OF PATHOLOGIC CASES

NAME	DIAGNOSIS	HEMATOCRIT READINGS				CURVE
		PEP 0.5	CENT 0.6	SODIUM 0.7	OXALATE 0.8	
B	Secondary anemia	18	32	30	28	N
C	"	28	30	27	25	N
D	"	33	35	32	29	N
C	"	11	34	30	25	N
W	Obstructive jaundice	56	49	44	41	IR
J	"	57	53	47	44	IR
B	Nonobstructive jaundice	23	58	63	57	IF
R	Ruptured gall bladder bile in peritoneal cavity	52	47	45	45	N

N normal curve IR curve of increased resistance IF curve of increased fragility

The results of the hematocrit method for the estimation of red cell fragility give substantially the same results as do any of the methods using hypotonic saline. A normal curve is obtained in anemia (Fig 3 D), and curves of increased resistance in obstructive jaundice (Fig 3 B). In a single instance of childhood nonobstructive jaundice a curve of increased fragility was obtained (Fig 3 C). In practically every normal subject (48 out of 50) a normal result was obtained the abnormal curves both pointing to a slight increased resistance of the cells in question.

It is not necessary as it is when using venous blood and hypotonic saline, to include a normal control in the test. The diluting fluids may be prepared accurately enough to give comparative results, and on the whole, the operation of the hematocrit is so standardized that the limit of error in a carefully performed test is negligible.

CONCLUSIONS

The hematocrit method for the estimation of corpuscle fragility has the following advantages over the other methods in use at the present time:

- 1 Capillary blood obtained from either the finger or ear, is used thus eliminating venipuncture.

- 2 Fragility tests may be made without difficulty on infants and children. This usually has been inconvenient due to the difficulty of obtaining several cubic centimeters of blood in such cases.

- 3 Only four hypotonic solutions are used in the test, in place of the dozen or more where saline is used. Furthermore the sodium oxalate solutions need not be made up freshly and are stable.

- 4 The test is completed within thirty minutes. The time element is thus reduced considerably.

- 5 A normal control is unnecessary, due to standardization of materials and technique.

- 6 The interpretation of results is easier and more accurate than is the case using a two figure index. Results are expressed as a "fragility curve" comparable to a curve of a "glucose tolerance test."

- 7 Results with the hematocrit method are constant.

SUMMARY

An hematocrit method for the estimation of red cell fragility has been described, using four hypotonic (0.5, 0.6, 0.7, 0.8 per cent) solutions of sodium oxalate, four hematocrit pipettes, and capillary blood. Results are expressed in a fragility curve. This method possesses several advantages over the methods using venous blood and hypotonic saline. Results with the test are constant and normal in healthy, normal subjects, and vary as do the results of the other methods in disease.

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THE BUNSEN VALVE IN BLOOD-UREA DETERMINATIONS*

BY F P BROOKS, PH D, CHAPEL HILL, N C

EXPERIENCE has shown that the Folin-Wu method for the determination of blood urea presents many technical difficulties. That these difficulties have not yet been satisfactorily overcome is evident from the many modifications appearing in the journals and from the attempts being made to find methods offering less difficulties of manipulation. The chief troubles with the method seem to have been two in number. First, the uricase solutions have caused foaming, and second, the distillation has been attended by frequent aspiration of the receiving acid back into the distilling tube, due to the sudden cooling of the distilling tube by air currents or variations in the heat of the burner.

Of the many modifications suggested, it appears to me that the one proposed by Johnson¹ is the simplest and best solution of the problem. This method eliminates the back aspiration by closing the end of the delivery tube with a flutter valve and allows the operator to center his attention upon the prevention of foaming. In considering this method it occurred to me that the difficulty of finding flutter valves of the proper size might be obviated by

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¹Johnson, S Lloyd. JOUR LAB AND CLIN MED, 1924, ix, 860.

the use of a device, namely, the Bunsen valve, which can be easily and quickly made by anyone with a small piece of thin walled tubing, a piece of glass rod, and a razor blade

The Bunsen valve is an instrument, already familiar to the chemist, consisting of a piece of rubber tubing preferably of about three sixteenths inch bore and with walls from one sixteenth to one eighth inch thick. One end of the tube is closed by a piece¹ of glass rod. One or more slits are made lengthwise in the sides of the tube close to the closed end. These should be made with a sharp razor blade and should be from one quarter to half an inch in length. This constitutes the Bunsen valve. When placed on the end of a bent glass tube as originally used by Folin Wu it affords a satisfactory flutter valve. When the contents of the distillation tube are boiled, the air and steam escape through the slits in the valve. Whenever any back pressure develops these slits close tightly and prevent the back suction of the acid

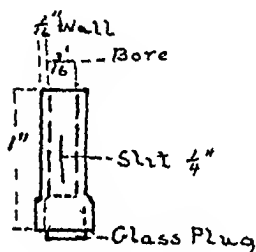


Fig. 1—Bunsen valve (actual size)

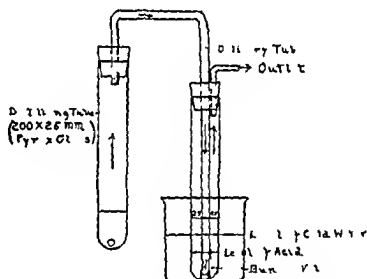


Fig. 2—Folin Wu urea distilling apparatus (according to Johnson) with the Bunsen valve attachment ($\frac{1}{2}$ actual size)

from the receiving tube. This device has the advantage over the flutter valve devised by Johnson of being quickly constructed from materials easily accessible to the average operator.

This device solves the problem of back aspiration and allows the heating of the distillation tube to be varied with the foaming. However, it has been my experience that this removal and exclusion of air has little or no effect upon the foaming. As a solution for this problem I have found that a small piece of fixing paraffin (M.P. 47-49) inserted in the distillation tube before heating is commenced, helps to overcome this problem. Before the contents of the tube reach the boiling point, this becomes liquid, and by surfacing the walls of the tube helps greatly to keep down foaming. Paraffin oil has been found to distill over and to interfere with 'Nesslerization' by causing clouding. Very little of this paraffin goes over and when the receiver and its contents are cooled the paraffin solidifies and rises to the top. 'Nesslerization' of such a solution gives a perfectly clear solution which can be compared in

NOTES ON LABORATORY TECHNIC*

By THOMAS B. MAGATH, M.D., ROCHESTER, MINNESOTA

BLOOD CULTURES

WHILE a great number of technical methods have been described for making blood cultures, none seems to fulfill the needs of everybody since each individual has certain problems which are not common to others. At the Mayo Clinic a number of methods of making blood cultures have been tried and the present one, which has now been in use for more than seven years and followed in more than 3500 cases, has proved satisfactory. The conditions which had to be fulfilled are as follows: (1) the method must be applicable

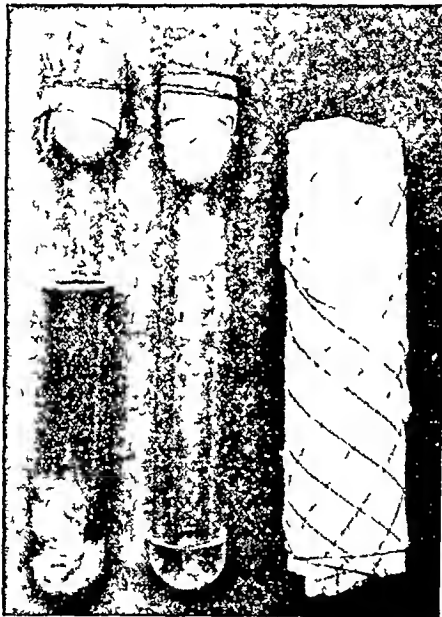


Fig. 1.—Blood-culture outfit: the towel contains a baked syringe and its needles; the test tubes contain respectively glucose-brain broth and sodium citrate solution.

in all types of septicemia, (2) reports must be rendered with reasonable promptness, (3) there must be an opportunity to decide whether or not a positive culture is due to contamination, (4) a large number of cultures have to be taken, (5) the patient may be ambulatory and the blood be drawn in the laboratory or, (6) the blood may have to be drawn at the bedside in private homes or in hospitals.

The following equipment is necessary and is prepared as follows:

1. A 20 c.c. Luer syringe and two needles, one No. 19 and one No. 21, are wrapped in a towel and baked at 170° C. for an hour.

*Section on Clinical Pathology, Mayo Clinic.
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2 Two test tubes (25 by 20 cm) are used one containing 5 c.c. of a 1 per cent solution of sodium citrate the other containing dextrose brain broth. The brain broth is put into the tube to a height of 15 cm. and both tubes are plugged with gauze and cotton stoppers, the tops being wrapped with brown paper which is secured with a rubber band (Fig. 1). Both tubes are autoclaved. This paper covering does away with the necessity of flaming the tubes at the time of drawing the blood.

Blood is drawn from a suitable vein in the arm after the skin has been prepared by thorough washing with alcohol. Other preparation has been found unnecessary and of no advantage. From 15 to 20 c.c. of blood is secured in a dry syringe, from 3 to 5 c.c. being placed immediately in the brain broth tube and the rest in the solution of citrate. The tubes are brought to the laboratory, the brain broth tube being immediately placed in the incubator and

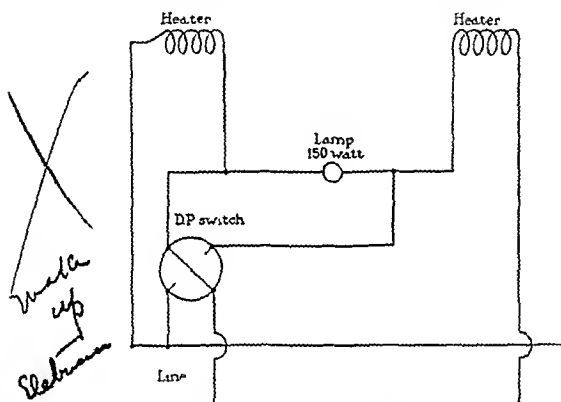


Fig. 2—Microscope lamp showing slots for screens

blood agar plates being made from the citrated blood. Hormone agar is used as a base, and four plates are poured, the first containing 0.5 c.c. of blood, the second 1 c.c. of blood, the third 2 c.c. and the fourth 3 c.c. The plates and the tubes are examined at the end of twenty-four hours; if negative they are put back in the incubator for another twenty-four hours, the report being made at the end of forty-eight hours. The brain broth tube is then set aside in the incubator and examined at weekly intervals for three weeks. Recently we have been employing a moist chamber in the incubator and holding the plates prepared from certain types of cases for a week. By distributing the blood on plates in this fashion and comparing them with the brain broth tube, it is possible in most cases to recognize contamination; moreover the method permits of a quantitative report on the number of colonies for each cubic centimeter of blood. The brain broth tube has enough liquid in it together with the brain tissue to bring about reduction in oxygen tension,

thereby increasing the possibility of growing certain streptococci in Rose now's opinion. The method has proved satisfactory in culturing hemolytic and nonhemolytic streptococci, colon bacilli, typhoid bacilli, anthrax bacilli, pneumococci and influenza bacilli. It is a simple, direct method, with a minimum of labor for the technician and gives reliable results.

A SIMPLE MICROSCOPE LAMP

With the increasing use of binocular microscopes and the developing of laboratories in the heart of business sections in cities, artificial light has come to be an important problem to the microscopist. Most of the microscope lamps on the market require special bulbs that are short-lived and which do not give sufficient light, the large types are entirely too expensive to be used in laboratories requiring large numbers of microscope lamps. Following is a description of a lamp which cost \$1.50 and which could be made in large

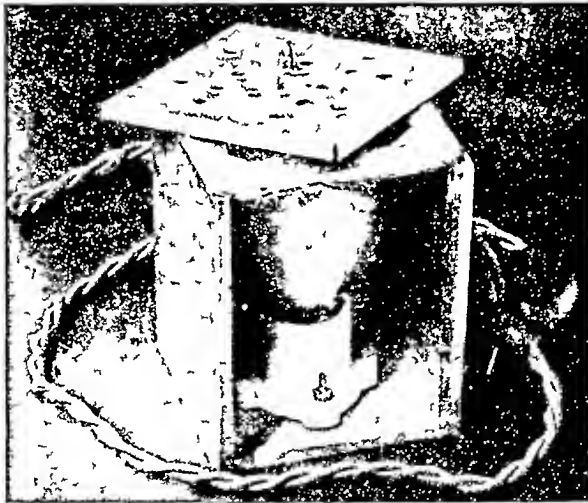


Fig. 3—When the switch is in the off position the heaters are in series and the temperature is about 46° C. when in the on position the heaters are in parallel and the water boils. The plug is withdrawn to disconnect.

quantities for less and is more satisfactory than any light yet used. It is made of polished tin, its dimensions are 10 by 10 by 12.5 cm. In the bottom an ordinary porcelain bulb-socket is secured by two screws. The front is made with two grooves into which are fitted glass screens. The source of light is an ordinary frosted Mazda 40 or 60 watt bulb. The top is movable and is ventilated, as well as the back. Electric light wires protected by the usual insulation are connected with the socket (Fig. 2). The screens are prepared by staining a developed photographic plate. For examining unstained material, such as fresh stool, a screen stained with a dilute solution of Orange G makes an excellent light. Daylight glass can be purchased, making a white light for use with stained material, and any shade of blue may be obtained by staining a developed photographic plate with methylene blue. These screens last for years and give excellent results.

TABULATION

TEST	AMOUNT OF BLOOD cc	DISPOSITION OF BLOOD
Wassermann	4	In a Wassermann tube
Coagulation time	1*	Rinse syringe and Wassermann tube with normal solution of sodium chloride test every 30 seconds
Calcium coagulation time	1	As coagulation test but after rinsing tube add 6 capillary drops of 0.5 per cent calcium chloride to the tube
Prothrombin	4	Add 4 cc blood to graduating centrifuge tube in which has been placed 0.5 cc of 1 per cent potassium oxalate solution. Invert tube to mix
Retractility of clot	1	In a Wassermann tube
Fragility	2	Add one drop of blood to each of 12 tubes containing sodium chloride in varying concentrations
Grouping	4	Add 3 drops to a Wassermann tube half full of 2 per cent sodium citrate solution the remainder in a dry tube
Widal and other agglutination tests	4	In a Wassermann tube
Methemoglobin and sulphemoglobin	3	Add to 5 cc of distilled water shake
Serum bilirubin	8	Rinse syringe and centrifuge tube with normal solution of sodium chloride first
Sugar	6	In large oxalate tube (1 cc of 3 per cent potassium oxalate heated to dryness) shake well
Urea and creatinine	8	As for sugar
Uric acid	8	As for sugar
Inorganic phosphorus	12	As for sugar
Lipoid phosphorus	8	As for sugar
Chlorides	6	As for sugar
Calcium and magnesium	8	Centrifuge tube and syringes must be rinsed thoroughly in distilled water
Carbon dioxide combining power	6	In tube containing dry oxalate and mineral oil shake
Cholesterol	3	Add to bottle containing 25 cc ether and 75 cc alcohol shake
Blood culture	15	2 to 5 cc in large brain broth tube and remainder in large test tube containing 5 cc 1 per cent sodium citrate sterilized Use baked syringe
Volume index	10	Graduated centrifuge tube in which is placed 2 cc of 16 per cent sodium oxalate

Exact measurement

A METHOD OF KEEPING MELTED AGAR

For more than four years we have used two Universal water heaters capacity about one quart each for keeping agar melted in the laboratory. The two heaters are so wired with a switch and a lamp that in one position of the switch, water is boiled in both containers and at another position of the switch, the temperature is reduced to about 46° C which keeps the agar melted. The diagram of the wiring is given in Fig 3 where it can be seen that the light bulb and the heating elements are used for resistance. During the four years the only repairs have been replacement of the lamp once and

of the small fuses once each. The apparatus is in constant use, burning all day, and melted again at the right temperature is always on hand.

DIRECTIONS FOR TAKING BLOOD SPECIMENS FOR VARIOUS TESTS

The accompanying tabulation has been found useful when placed in the bleeding rooms where blood is being taken for various tests, it is also mounted in the tray which is used in collecting samples in the hospitals. It leaves no doubt in the technician's mind as to the exact method of taking blood specimens for the various tests which may be performed in the laboratories. A slight modification of the table might be useful in almost any laboratory.

A METHOD FOR HANDLING LARGE NUMBERS OF SLIDES IN STAINING FOR THE BACILLUS TUBERCULOSIS

Two possible methods of staining for bacillus tuberculosis in routine laboratories are available, one consists of staining each slide separately, the other of staining slides in bulk. The objections to staining in bulk have usually been that more stain is used than necessary and that the constant heating of the stain causes precipitation, on the other hand, the objection to the slide-staining method is that the staining is more laborious and time-consuming and is usually messy. The tendency of the slides to become scorched together with the constant necessity of watching them, makes the method not an ideal one. With a smaller vat, less stain can be used, and if the procedure is properly carried out, the results are extremely satisfactory. The staining vat which we have used is a Vollrath refrigerator pan 15 by 15 by 7.5 cm. It is kept on an electric hot-plate and is heated and kept at a temperature of 80° C while the staining is in process. The decolorizing and counterstaining reagents are kept in glass refrigerator dishes purchased at a novelty store at a cost of 10 cents each. These dishes have covers and measure 12 by 12 by 5.5 cm. In our laboratory three methods of staining are carried on simultaneously. The first consists of heating with carbolfuchsin for five minutes, decolorizing in 10 per cent nitric acid in water and then in 95 per cent alcohol and counterstaining with methylene blue. The second varies from the first in that 5 per cent sulphuric acid in 95 per cent alcohol is used as a decolorizing reagent. The third is the Schultze-Tigges method which consists of staining for one minute with carbolfuchsin, decolorizing with fresh 10 per cent sodium sulphite and counterstaining with picroic acid. The method I have described is suitable for handling a large number of slides by the three methods simultaneously. The important feature is the carrier for the slides (Fig. 4). It is made of aluminum 2.5 mm thick, the frame is 6.25 cm wide and 10 cm long. An aluminum guard made of 1.5 mm material is attached to each side and bent parallel to the side. From one guard to the other measures 7.8 cm. The aluminum sides that hold the slides are slotted for thirty slides. Occasionally one can find on the market an aluminum comb which has the teeth set at the exact distance to hold the slides. The ends of the carrier are suitably shaped to carry a wire spring handle (not shown in the illustration) and are marked with numbers serially to indicate the batch. The slides are dropped in in order

after being numbered with a diamond pencil and are stained in bulk. If 100 slides are stained each day, the carbolfuchsin should be changed once a week, the other reagents will last for at least a week and the counterstaining reagents longer. The results have been extremely satisfactory for a long time and this method eliminates much of the drudgery of routine staining by the single slide method. The slide carriers were originally designed by Whitman for use in tissue staining and improved upon by Lundquist but since the aluminum withstood the acid used in staining bacilli of tuberculosis the method has been developed for that use.

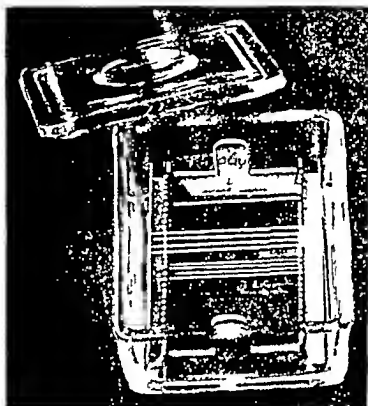


Fig. 4—Slide-carrier in staining vessel

METHOD FOR MAKING TISSUE CULTURES

The most common way of taking cultures of tissues those that have been removed surgically or at the necropsy table, is to sear the surface with a hot spatula. This is often unsatisfactory and fails to give uncontaminated cultures. A solution to the problem is offered by the use of the Lenk Improved Automatic Torch. This gives a direct very hot pencil flame. It operates with alcohol and is ready almost immediately after lighting, the instrument is simple, positive and inexpensive. The flame is also suitable for flaming the mouths of test tubes and for pipettes. The percentage of contaminations will drop immediately upon its use.

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COLLOIDAL GOLD SOLUTION*

THE PREPARATION OF GOLD SOLUTIONS AND THEIR TITRATION WITH PERMANENT H-ION CONCENTRATION STANDARDS

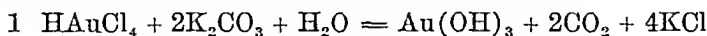
BY VINCENT CHRISTINA AND CLARA S. GREEN, NEW YORK CITY

INTRODUCTION

ONE of the most important items in the Lange gold test for the differentiation of cerebrospinal syphilis is the necessary neutrality of the gold solution employed. It has been found extremely difficult to prepare strictly neutral solutions with consistently satisfactory results. We suggest a slightly modified mode of preparation and a simple and reliable method of titration and correction of the colloidal gold solution.

The methods generally accepted as the best for obtaining bright red, completely clear and stable solutions, are those of Zsigmondy,¹ without oxalic acid, and of Miller and his coworkers,² with oxalic acid. We have found the use of oxalic acid unnecessary. The investigations of Zsigmondy and his coworkers show that if it is desired to obtain a fine-grained, bright red, clear, and stable solution, the velocity of formation of nuclei must be taken into consideration. The importance of the velocity of formation of the nuclei has been shown in the formaldehyde reduction method. If reduction takes place in less than a minute, we obtain bright red, clear solutions due to the large number of particles, while if the reduction of the gold is retarded, we obtain highly fluorescent and unstable solutions ranging in color from blue to purple. Zsigmondy has also found that the presence of ammonia reduces the velocity of formation of nuclei, and that CO₂ interferes with the production of red, fine-grained solutions. Therefore, every precaution must be taken to insure their absence.

In the formaldehyde method we start with acid gold chloride and a slight excess of K₂CO₃, since it is known that reduction takes place best in a slightly alkaline medium. The following reaction³ occurs:



Since the CO₂ formed interferes with the production of fine-grained solutions, the heating is continued after adding K₂CO₃ until all of the gas is removed.

To overcome the interference of dissolved ammonia and CO₂ in water, conductivity water is used in this laboratory. The titration of gold solutions with alizarin, the method most frequently employed, is unsatisfactory for sev-

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eral reasons Alizarin as an indicator has too wide a range, and the color change after the addition of this indicator is not sharp enough with small increases of acid or alkali to warrant its use

The hydrogen electrode method is indubitably the most accurate, but it is time consuming and too expensive for clinical laboratories The colorimetric method of determining P_H value is simple and rapid, provided that correct buffer and indicator solutions are at hand However a certain amount of difficulty lies in the fact that these solutions are unstable, and their repeated preparation is difficult and consumes much time

We have found Taub's⁴ permanent standards in a slightly modified form satisfactory Our permanent standards prepared according to this method were checked against the Lovibond tintometer, and Hynson Westcott and Dunning organic standards The gold solution which was standardized with our inorganic standards was also checked against Hynson, Westcott and Dunning organic standards and the hydrogen electrode

PROCEDURE

Glassware—Use new Jena glassware which has been thoroughly washed with soap and hot water Then soak in hot dichromate cleansing mixture overnight, rinse thoroughly under tap water next with distilled, and just before use with conductivity water

Conductivity Water—To every liter of distilled water (from a Stokes still) add 1 gm of chromic acid and distill in an all glass distilling apparatus avoiding use of rubber stoppers and connections To every liter of water distilled over chromic acid add 1 gm of barium hydrate This may now be kept as a stock supply to be redistilled immediately before use

Reagents—Merck's gold chloride acid yellow—1 per cent solution Merck's potassium carbonate C P—2 per cent solution Merck's neutral formaldehyde solution—1 to 40 dilution These reagents are made up with fresh conductivity water

Preparation of Colloidal Gold—Heat 1 liter of fresh conductivity water to 60° C at this temperature add 10 c c of 1 per cent gold chloride solution and 8 c c of 2 per cent potassium carbonate solution Continue the heating until the temperature reaches 92° to 95° C then remove the flame and add 5 c c of formaldehyde solution, 1 to 40 dilution Stir briskly until a full bright red color develops

The necessity of brisk and even stirring must be emphasized, otherwise reduction will not be uniform After the solution has cooled to room temperature, preferably after standing overnight the P_H of the gold solution may be determined

TITRATIONS

The permanent inorganic standards are prepared with slight modification, according to Taub The following solutions are necessary for a P_H range of 6.6 to 7.4

N/2 cobalt chloride 59.497 gm $CoCl \cdot 6H_2O$ per liter of 1 per cent HCl

N/2 ferric chloride 45.054 gm $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ per liter of 1 per cent HCl

The indicator is prepared as follows. Dissolve 1 gm of phenol red in 52 cc of neutral 95 per cent alcohol, add 28.5 cc of N/100 NaOH and sufficient conductivity water to bring the volume up to 250 cc. Store the indicator in a Pyrex bottle.

MATCHING BLENDS

Table I indicates the amounts of the chlorides necessary to give the correct colors for the standards.

TABLE I

P_H	CO (cc)	FF (cc)	CONDUCTIVITY
			H_2O (cc)
6.6	1.8	6.5	11.7
6.7*	2.1	5.95	11.95
6.8	2.4	7.4	12.2
6.9*	2.95	4.15	12.9
7.0	3.5	2.9	13.6
7.1*	4.25	2.1	13.65
7.2	7.0	1.3	13.7
7.3*	6.1	0.8	13.1
7.4	7.2	0.3	12.5

*Our own additions

The solutions are placed in test tubes of uniform length, diameter, and thickness. They are then sealed into ampoule form and sterilized in the autoclave at 15 pounds pressure for fifteen minutes to insure complete sterility. The tubes are allowed to cool overnight.

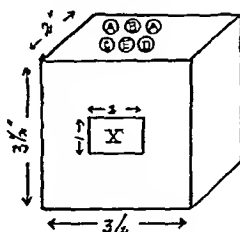


Fig 1—A equals perforation with frosted glass attached in rear

A comparator block (Fig 1) is used to make the P_H determination. In making determinations the test tubes should be of the same diameter and thickness as the uniform tubes used for standards.

To 10 cc of the gold solution, add 0.15 cc of indicator and mix well. Place this tube in Hole E. In Hole B place a tube with distilled H_2O . In Holes A, A, place tubes containing gold solution without indicator. Holes C and D are for the P_H standards. If the gold solution does not match the 7.0 P_H standard, then N/100 NaOH or N/100 HCl is added, as the case may require. If the solution shows a P_H below 7.0, alkali is used for the titration, if it is above 7.0, then acid is employed. After each addition of acid or alkali to the gold solution, the contents of the tube are thoroughly mixed by inverting the tube several times.

When the unknown gold solution is matched with the 70 standard, the necessary correction is made for the entire volume of gold on hand. It is a practice in this laboratory to make a P_H determination on our gold solutions immediately before use as they have a tendency to become slightly alkaline after two weeks' standing.

After correction, we test the neutrality of the solution by adding 17 cc of 1 per cent NaCl to 5 cc of the colloidal gold. The gold will be completely precipitated in an hour. It must give a typical curve with a known paretic spinal fluid, and no reaction, or a red blue change at the most in the first or second tube, with a negative spinal fluid.

SUMMARY

1 A slight modification of Zsigmondy's method of preparation of colloidal gold is described, wherein conductivity, instead of triply distilled water is used, in addition to an increased amount of alkali.

2 The titration of the solution is accomplished with phenol red as an indicator, and the P_H is determined with permanent inorganic standards.

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A NEW AUTOMATIC PIPETTE*

INCORPORATING A MECHANICALLY OPERATED TWO WAY VALVE

BY F W HARTMAN, M D, DETROIT, MICH

IN THE large laboratory the problem of pipetting accurately measured quantities of fluids entails much monotonous yet exacting labor. This applies particularly in the set up of the Wassermann test and in the filling of ampoules. A number of automatic pipettes have been proposed, notably those of Woodyatt, Lorenze, and Laird. As in all automatic pipettes these depend upon a two way valve, and in each of those mentioned the valve is actuated by the flow of fluid through it or by the flow of fluid plus a spring. In the use of these pipettes it was found that they needed frequent adjustment, especially when reagents such as amboceptor, antigen, and red cell suspensions were used or when the speed of operation varied. The height of the supply bottle and delivery tip when modified also produced variation in the delivered amounts.

It occurred to us that valves actuated by the flow of fluids through them could not be expected to give uniform or accurate delivery under varying con-

ditions, particularly when the motor speed was altered or when fluids tending to make the valves stick, as reagents containing serum or lipoids, were handled

To avoid variations in the delivered amounts under varying operating conditions, it seemed essential to have the valve operated mechanically with

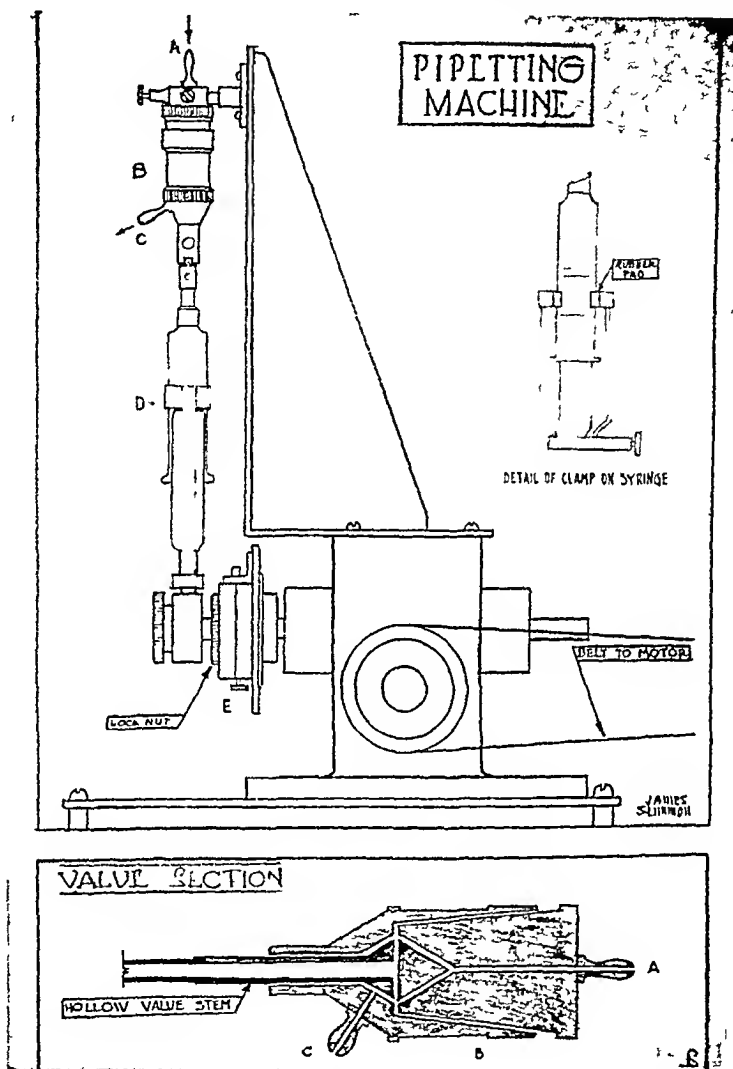


Fig 1

the action of the syringe plunger, thus making the valve independent of the speed and force of the fluid current

An apparatus incorporating a mechanically operated valve which is still automatic was finally evolved. The principle depends upon the attachment of the valve core to the barrel of the syringe then actuating this barrel by friction either from the plunger or in combination with an outside friction pad

A cross section of the valve is shown in the lower illustration (Fig 1). The valve seat block B is made in two parts which are held together by a

slip lock, so that they may be readily separated and the valve seats cleaned. The valve core is a double cone with a stem extending from the block with an attachment at the end for a Luer loc (Beeton Dickinson) syringe. From the sides of the cone, openings connect with the hollow valve stem. When the valve is assembled, as shown in the illustration, and the lower cone of the core is seated by pulling on the valve stem, fluid may enter, flowing around the upper part of the cone and into the valve stem through the openings in the sides of the core. If the cone is pushed against the upper valve seat, the intake is securely blocked and fluid in the valve stem may be forced out the lateral openings in the core around the lower cone and out at *C*.

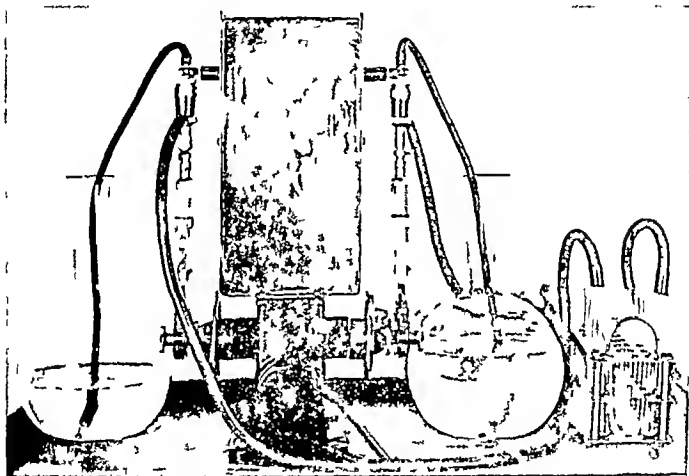


Fig. 2

The valve attached to a Luer loc syringe and the whole mounted with gear and adjustment is shown in the upper illustration (Fig. 1). The adjustment *E* controls the length of the piston stroke and is made with screw and lock nut. This adjustment when used with a 5 cc syringe will allow delivery of from $\frac{1}{10}$ to 5 cc. At slow speeds and with a 10 cc syringe the larger amounts may be delivered.

The barrel of the syringe and the attached valve core is actuated by the friction of the plunger with each upward or downward stroke, and this is supplemented by a clamp which is attached with the syringe piston to the drive shaft below. The upper portion of the clamp ends in two rubber friction pads which make readily adjustable friction against the syringe barrel.

The machine is driven by a one eighth horse power motor, the speed of which should be adjustable. In operation, the fluid is taken up from a flask

on the table through a tube to *A*, into the syringe and out at *C*, being delivered through pressure tubing to test tubes or ampoules

Smaller amounts, that is $\frac{1}{10}$ to 1 cc, may be delivered at rates of from 100 to 200 per minute. Larger amounts up to 5 cc may be delivered at rates of 50 to 100 per minute. With a still larger syringe and a larger valve 50 or 100 cc amounts may be handled rapidly. A double pipette is easily arranged to run with the same motor and gear (Fig 2). With this double arrangement two operators are necessary, but twice the amount of pipetting may be accomplished. It will be noted that the syringe, valve, and tubing are quickly detachable, so that they may be sterilized and replaced without disturbing the adjustment.

As to accuracy, if the temperature is controlled, the variation is less than one-half of 1 per cent. Although checked many times a day with different solutions, the variation is found negligible. The apparatus was not designed for a pressure pump, but it will deliver accurately against a pressure of 300 mm of mercury when the friction clamp is properly adjusted. Necessary adjustments under usual working conditions are made on an average of once a month, and these are usually after cleaning or replacement of syringes. When changing from one reagent to another, saline is washed through the apparatus for a period of five minutes. Care is taken to flush the machine for the same period before leaving it for the night. Cleaning is readily accomplished by detaching the syringe and disassembling the valve block. If there is an accumulation of material on the valve seats or core, this may be removed with dilute cleaning solution.

SUMMARY

A positive accurate automatic pipette incorporating a new mechanically operated two-way valve is presented. The use of the machine in Wassermann work gives greater accuracy than obtained by hand pipetting, saves much time, and eliminates most of the monotonous drudgery of putting in the salt solution and various reagents. Nearly two years operation in our own laboratories show repairs and replacements rarely necessary.

The mechanical perfection of this machine was made possible by Alfred Schmitt, instrument maker of the Henry Ford Hospital, who has built the original models.

The complete apparatus is manufactured by the Detroit Laboratory and Hospital Supply Company, 8019 Third Avenue, Detroit, Michigan.

Write for price a new automatic pipette

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A NOTE ON THE REFLEX NATURE OF STRYCHNINE CONVULSIONS*

BY SEYMOUR J COHEN, M.S. M.D. CHICAGO

THE fact that strychnine causes convulsions which are reflex in origin has been known for years, but the actual demonstration for class work has been quite unsatisfactory. As early as 1846 Meyer¹ showed that on cutting the posterior roots of all the spinal nerves in frogs and then injecting strychnine, no convulsions appeared. When the central end of the nerve was touched marked convulsions occurred thus showing that a sensory stimulation is necessary to initiate the convulsions. Hering repeated this experiment but put the strychnine directly on the cord. Again there were no convulsions unless the animal was severely shaken or the central stumps of the nerves were touched. These experiments are very difficult to demonstrate to the students in a laboratory.

Paulson,² in 1889, showed, by painting the skin of a frog with cocaine or chlorotone, thereby paralyzing the sensory nerve ends injection of strychnine was not followed by convulsions unless the animal was strongly stimulated. Meyer¹ used 30 per cent hydrocyanic acid to paralyze the nerve endings with the same results. These experiments also show that a sensory stimulation is necessary to initiate a strychnine convulsion. They are objectionable however, because if too much cocaine is absorbed by the skin and reaches the circulation, it may accentuate these convulsions and defeat the point of the experiment, while too much chlorotone may depress the spinal cord and consequently in itself abolish the convulsions. Hydrocyanic acid is much too dangerous to use in the laboratory.

All the above experiments are open to criticism being either too difficult to perform in a classroom or else some of the drugs may be absorbed and modify the experiment.

A simple method of showing conclusively that the convulsions caused by strychnine are reflex in origin is as follows. In a decerebrated frog one sciatic nerve is exposed and cut. Then $\frac{1}{2}$ mg. of strychnine is injected into the ventral lymph sac. The leg with the cut sciatic nerve is touched with a probe no convulsions occur. The other leg with the nerve intact is touched and is followed by marked convulsions throughout the body of the frog.

Inasmuch as the impulse cannot travel centrally in the cut sciatic nerve no stimulus is received by the cord and hence no convulsions. This simple experiment can easily be demonstrated and shows quite conclusively the reflex nature of strychnine convulsions.

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From the Laboratory of Pharmacology University of Illinois College of Medicine Chicago

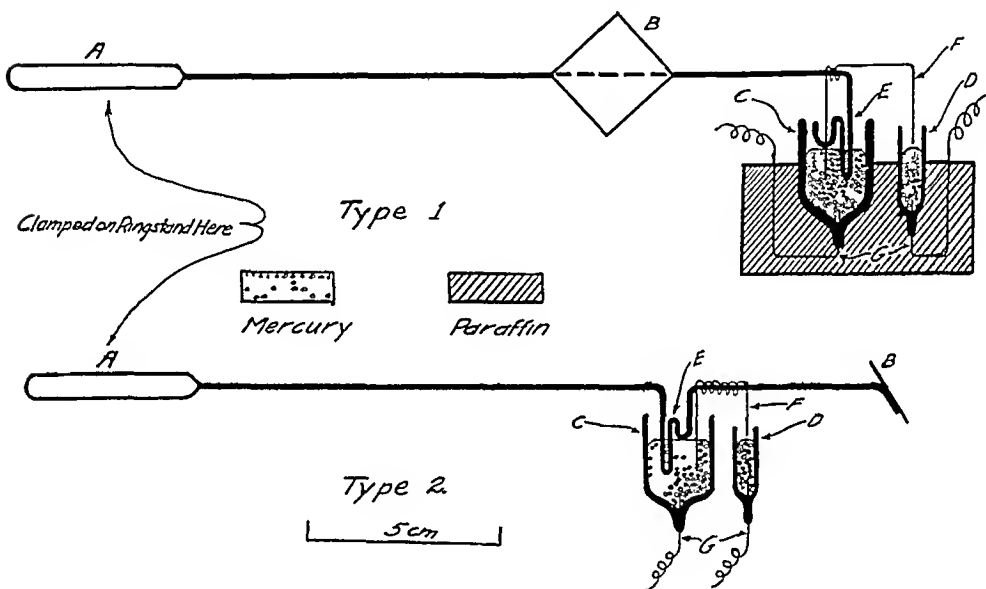
Received for publication April 6 1907

A SIMPLE DROP COUNTER*

BY EDGAR J POTH, PH D, BALTIMORE, MD

A SIMPLE, easily adjusted, and efficient device for counting drops is often needed in biologic work. In the accompanying illustration two types of such an arrangement are given.

A, in each case, is a glass rod or tube drawn out and bent as shown. B, in Type 1, is a cover slide made fast to the rod with beeswax, sealing wax, or DeKhotinsky cement. In the case of Type 2 only a part of a cover slide is used for B so as to keep the weight beyond E a minimum. C and D are two mercury cups with sealed-in platinum wires (G) and containing mercury. The wires (G) are leads to the usual electric signal system assembled so as to write on a



revolving drum. F is a platinum wire, fastened with beeswax or similar material, and serves to complete the electrical circuit at D whenever a drop falls on B.

The important part of this arrangement is at E. The buoyant action of the mercury on the longer shank of the rod, which is submerged, keeps the rod under stress and, due to the viscosity of the mercury, eliminates vibrations in the sidewise or horizontal directions. The shorter shank with its lower bend adjusted so as to rest just on the mercury surface arrests up and down or vertical vibrations.

The final adjustment is made by varying the height of the mercury in C and D. The result is an easily adjusted, rapidly acting, and fully damped device for counting drops, which at the same time is simple in construction and entirely reliable as to accuracy.

*From the Department of Pharmacology, Johns Hopkins University.
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NOTE ON THE VOLHARD HARVEY METHOD FOR THE ESTIMATION OF CHLORIDES IN URINE*

BY WILLIS H. JEFFERY, PROVIDENCE, RHODE ISLAND

IN THE course of class work in the quantitative analysis of urine for chlorides, some difficulty has occasionally been encountered in the clear perception of the end point in the Volhard Harvey method. Such difficulty is usually found to be associated with a very concentrated twenty-four hour sample, one in which the volume is six hundred cubic centimeters or less. Such a sample often has an amber color which tends to mask the appearance of the color of the ferric ammonium thiocyanate. In cases of this sort it has been found helpful to dilute the five cubic centimeters to fifty or more cubic centimeters.

But a hindrance which cannot be ascribed to this source has recently been noted. A particular sample of urine of average volume and light straw color gave a brownish violet color when treated with the reagents employed in this method. Subsequently, it transpired that the color was due to the acidified indicator alone. Then it seemed of interest to learn whether other ferric salts would also give the color. When saturated ferric chloride solution was added to some of the sample a purplish red color unobscured by any precipitate, was obtained. The color was so like the false positive result of the Gerhardt test for acetoacetic acid that it was at once suspected that the subject had taken aspirin just prior to the collection of the sample. Upon inquiry it was learned that he had taken two aspirin tablets on the evening before the collection of the sample was started.

Further trials with aspirin on different subjects were made and it became evident that the ingestion of one aspirin tablet was sufficient to give the above-mentioned color with the acidified indicator, over a period of twenty-four to thirty-six hours. In order to insure the observation of a sharp end point, the employment of an extra dish containing an untitrated mixture to serve as a control, together with the first rough titration, was found indispensable.

AN IMPROVED TECHNIC FOR DEMONSTRATING EXPERIMENTAL JACKSONIAN EPILEPSY*

By LESTER R. DRAGSTEDT, PH D, M D, AND CARL A. DRAGSTEDT, PH D, M D,
CHICAGO, ILL

THE following is a modification of a technic in use in the Physiologic Laboratory of the University of Chicago for demonstrating the effect of an electric stimulation of the motor cortex of experimental animals. The symptoms resemble those seen in Jacksonian epilepsy. Dogs are preferably used. The animal is anesthetized and the field prepared with careful aseptic precautions. A median incision is made over the vertex, and the skin is widely reflected. The temporal fascia and muscle is divided along its origin and peeled laterally from the bone. Two small holes, about two millimeters in diameter, are drilled through the calvarium over the motor area, preferably one hole on either side. Electrical binding posts which have been previously sterilized are screwed into the holes, the brass screws penetrating the dura. Slits are then made in the temporal muscle and skin over the posts to permit these to extend to the exterior. The incision is closed. When the animal recovers from the anesthetic, wires leading from the secondary of an inductorium may be connected with the binding posts extending into the motor cortex and direct electrical stimulation with varying strengths of current may be obtained. It is best not to attempt to keep the animal for more than a day or so as infection invariably occurs along the course of the electrodes.

A METHOD FOR STUDYING THE SECRETION OF URINE IN EXPERIMENTAL ANIMALS†

By CARL A. DRAGSTEDT, PH D, M D, AND LESTER R. DRAGSTEDT, PH D, M D,
CHICAGO, ILL

THE chief objections to the usual methods of studying the secretion of urine in animals both for experimental investigation and in laboratory exercises for didactic purposes are first, the trauma incident to the operative procedures required for placing the cannulae in the ureters, which will often inhibit the secretion of urine for a varying period, and second, the toxic effect of the anesthetic on the kidneys. The following method permits of a very accurate study of the urinary secretion in animals under practically normal conditions. During the course of an experimental investigation of the surgery of the urogenital tract it was found that dogs will survive the produc

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tion of an artificial exstrophy of the bladder for long periods of time without developing kidney infections. These animals serve admirably for a study of the physiology of urinary secretion, the action of diuretics, and tests for kidney function. The operative procedure is not difficult. Females are selected for this work, a median incision is made just above the symphysis pubis and the bladder is secured and separated from its peritoneal attachments. The urethra is divided and closed, the bladder opened and the bladder wall trimmed away except for a small circular area which contains the orifices of the ureters. This area is stitched into the abdominal wall on closure of the incision. The wound heals readily and animals prepared in this fashion can be kept in the laboratory indefinitely without requiring any special attention. It is possible to secure the secretion from either ureter separately by means of cannulae or from both by means of an inverted funnel strapped to the abdominal wall. The animal should be secured in a wooden frame during the collection of all samples.

Erratum

In the article by Blumberg entitled 'Pathology of Intestinal Tuberculosis,' February, 1928 issue, the second sentence, eleventh paragraph, page 412, should read: The nonfermentative pasty, acholic, viscous stool, etc.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

TUBERCULIN VI Acid Hydrolysis of Tuberculin, Long, E. R. Am Rev Tuberc, May, 1926, *vol.*, No. 5, p. 441

A highly potent tuberculin prepared on synthetic medium was neutralized, and normal hydrochloric acid was added to 50 c.c. samples of it in such amounts that final concentrations of the acid were N/100, N/20, N/10, N/6, and N/3. These samples, with one which was neutral, were heated in the autoclave four hours at 120°. They were then neutralized with sodium hydrate, and to them salt and water were added in such amount that the final volume and salt content were the same in all. The first four samples (beginning with the one heated at neutrality) gave powerful skin reactions in a subject sensitive to tuberculin. A trace of reaction was given with the fifth sample and no reaction with the sixth. Trichloroacetic acid gave a precipitate immediately with the first four preparations, a trace of precipitate on standing in the fifth, and a smaller trace with the sixth. The trichloroacetic acid precipitates were filtered off, and all the filtrates were saturated with ammonium sulphate. A precipitate developed in all tubes. The first four precipitates of this series had tuberculin activity, but not the last two.

TUBERCULIN V The Effect of Proteolytic Enzymes on Tuberculin Proteins and the Activity of Tuberculin, Seibert, F. B. Am Rev Tuberc, May, 1926, *vol.*, No. 5, p. 393

Whenever the whole protein molecule (noncoagulable water soluble fraction) is attacked and changed to proteose and amino acids, as by the action of pepsin or trypsin with Na_2CO_3 , there is a complete loss in activity of the protein, as determined by negative skin tests in tuberculous guinea pigs. The effect is a direct result of enzymic action, since boiling of the enzyme before mixing destroys its ability to bring about these results.

Trypsin, in neutral solution, attacks first proteose, with no loss in activity.

Erepsin, an enzyme which is not able to attack the whole protein molecule, but which is able to act upon the peptide bonds already free in the proteose fraction, does not cause any loss in activity.

These experiments indicate that the active substance of the water soluble noncoagulable fraction used in this study is a whole protein molecule, or else it is so closely associated with this protein that its activity is lost when the whole protein molecule is disrupted.

TUBERCULIN VII The Evidence That the Active Principle Is a Protein, Long, E. R., and Seibert, F. B. Am Rev Tuberc, May, 1926, *vol.*, No. 5, p. 448

The active principle of tuberculin appears to be a whole protein. The experimental evidence for this is as follows. The active principle of untreated tuberculin is a colloidal substance which does not pass through animal and vegetable membranes, except in traces. A colloid giving all the usual protein tests may be precipitated by acids at its isoelectric point, $\text{pH} \approx 4.0$. This substance is highly active in the tuberculin test. Precipitation of protein is not complete by this method, however, and the filtrate, containing some protein, also retains tuberculin activity. Saturation of tuberculin solutions with ammonium sulphate precipitates all detectable protein. The precipitate is the most highly active of all tuberculin preparations obtained. The filtrate contains no protein and shows no tuberculin activity whatever. The most specific reagents for protein at our disposal, proteolytic enzymes, lessen or destroy

tuberculin activity at the same time that they disrupt protein present. As whole protein decreases and protein cleavage products accumulate in solutions of tuberculin subjected to proteolytic enzyme action, tuberculin activity decreases or disappears. When whole protein disappears on acid hydrolysis activity is lost. As long as however, as whole protein is present activity remains.

There is evidence that a protein product of smaller molecular weight than "whole protein" may be active. Active principle in tracea dialyzes. 'Proteoso' obtained by ammonium sulphate precipitation, after "whole protein" has been precipitated by trichloroacetic acid has some activity. These results signify that activity is associated primarily with the whole-protein molecule, but persists in spite of a certain amount of disintegration of the molecule.

CARCINOMA The Relation Between the Surface Tension of Blood Serum and Its Calcium Content in Carcinoma, Svehl, K. *Compt rend Soc de Biol*, April 23, 1926, xciv, 980

Surface tension was determined by Bang's torsion balance and expressed as dyn/cm. Calcium was determined by Kramer and Tisdall's method.

In carcinoma the average surface tension was 51.96 dyn/cm as compared to 63.0 dyn/cm in normal individuals.

The average calcium content in carcinoma was 0.185 mg per cent as compared to 0.232 mg per cent in normal individuals.

The lowering of the surface tension in carcinoma is in direct proportion to the lowering of its calcium content.

OZENA BACTERIOLOGY *Bacillus Ozena* Foetidae Perez and *Bacillus Proteus* in Ozena Michailoff. A. Bull. John Hopkins Hosp. September, 1926, xxxv No 3 p 158

Ozena is a chronic disease of the nose characterized by a mucopurulent discharge, crusts, a specific fetid odor, and atrophy of the turbinate bones.

A bacteriologic study of twenty-eight cases. The bacillus described by Perez was isolated in only seven cases. A bacillus of the proteus group was also found with sufficient frequency to warrant study of its connection with the disease.

Bacteriologic and immunologic studies are reported in detail and the author's conclusions are as follows:

Although the cultural and fermentative properties are insufficient to identify Perez bacillus as atypical *Proteus*, we are justified in thinking that it constitutes a subgroup which has lost some of its fermentative properties, a frequent occurrence with the most typical proteus and that *B. azarwanse liquefaciens* Shiga is a typical *B. proteus vulgaris*.

The agglutination and agglutinin absorption tests show Perez bacillus to be very closely related to *B. proteus*, Perez's is intermediate between the two species having agglutinative properties as strong for Perez bacillus as for *B. proteus*. Each bacillus of this group has a serologic individuality more or less close to the Perez or proteus species. Of the fourteen bacilli, no two are identical. Agglutinin absorption shows clearly that the motile strains, *B. ozenae liquefaciens* Shiga and *B. proteus vulgaris* as a rule have their coagglutinative branches reduced or removed when absorption is done with the motile bacillus, and vice versa they are more closely related to each other than to the non-motile Perez species and can be identified as belonging to the same group as *B. proteus*.

Complement fixation shows that the two varieties motile and nonmotile have a very close relation. Some Perez and Proteus bacilli have identical properties as antigens against a given serum and this reaction appears to show closer relationship than the agglutination or agglutinin absorption test. There is no correlation between the complement fixation and agglutination test.

The more specific flocculation reaction also shows the organisms to be very closely related, but only Perez II and Hofer's Perez bacilli have identical properties, while of these only the latter shows flocculation with *B proteus*

B proteus, *B Perez*, and *B ozaenae liquefaciens* have identical pathologic properties. Each produces the four different types of infection: (a) toxico-septicemic congestive and hemorrhagic lesions, (b) chronic pyemic, mucosal, and endothelial exudative and proliferative lesions, (c) local exudative lesions, and (d) special necrotic lesions due to complex pathologic processes.

All the strains studied produce a toxin identical with that of Hofer's Perez bacillus and *Proteus vulgaris*.

Cross immunization shows that Perez bacillus and *B proteus* have identical immunizing properties, protecting completely or producing sufficient protection to indicate their identity or very close relationship.

On the basis of the above conclusions it follows that the Perez bacillus is a member of the large group of *Proteus* bacilli.

The etiologic relation of the *Proteus* Perez group to *ozaenae fetida* has been suggested by many workers because of the frequent finding of these organisms in the nasal discharge of such cases. The *Proteus* group exhibits an affinity for the blood vessels and mucosae, has pathogenic power, producing chronic necrotic lesions and nasal ulceration and discharge. We never produced green crusts or atrophy of the turbinate bones in the rabbit, and therefore we cannot conclude that in human beings *B proteus* produces *ozaena fetida*, and that this bacillus acts as a primary etiologic agent. The frequency of its occurrence and the specific malodor found in the cultures show that *B proteus* is involved in the pathogenesis of *ozaena*, and whether implanted primarily or secondarily, is the cause of the fetor, discharge and ulceration.

TYPHOID COLON GROUP MEDIA. A Combination of Bismuth and Sodium Sulphite Affording an Enrichment and Selective Medium for the Typhoid-Paratyphoid Groups of Bacteria, Wilson, W J, and Blair, E M. Jour Path and Bacteriol, July, 1926, xxix, 310

To 100 cc of ordinary 3 per cent nutrient agar are added 5 cc of a 20 per cent solution of glucose, 10 cc of a 20 per cent solution of sodium sulphite (anhydrous) and 2 cc of liq bismuth et ammonii cit. The medium is boiled after the addition of the bismuth solution and poured out into Petri dishes. The surface is inoculated with watery suspensions of the bacteria to be tested or directly with thin typhoid stools. The material should be well rubbed into the surface. If the medium is heavily inoculated from a growth of *coli* on agar with a platinum loop, complete suppression of growth does not occur, whereas this is achieved after lighter inoculation with a watery suspension.

This medium inhibits the growth not only of ordinary strains of *coli* but also of those that reduce sulphites. It is not possible to say precisely what is the exact chemical constitution of the compound formed between bismuth and sodium sulphite which is active in the suppression of *coli*. A white precipitate forms on the addition of sodium sulphite to liq bismuth but this precipitate alone does not inhibit but combined with a certain excess of sodium sulphite it does.

A fluid enrichment medium consisting of 100 cc bouillon, 5 cc of 20 per cent glucose, 25 cc of 20 per cent sodium sulphite and 2 cc of liq bismuth has proved useful in the isolation of typhi from enteric stools. In the solid medium 0.5 cc of a 1 per cent solution of brilliant green can be added to each 100 cc of the medium. Strains of *coli* which reduced sulphites were not suppressed by brilliant green but they were inhibited by the bismuth sulphite combination.

Liquor bismuthi et ammonii cit in 1 in 500 to 1 in 1000 dilution alone without sulphite inhibits the growth of *Clostridium Welchii* in glucose agar.

RENAL FUNCTION The Dilution and Concentration Tests of Renal Function Pratt, J H Bost Med and Surg Jour July 29, 1926 exch No 5, p 203

The dilution and concentration tests give important information regarding kidney function. If an abnormal response is obtained the tests should be repeated once or twice after measures have been taken to remove disturbing extrarenal factors the chief of which are dehydration of the tissues and an excess of water in the organism. The tests should be employed in combination with the other functional methods of proved value and interpreted cautiously in the light of all facts that can be gained from the complete clinical study of the case. The tests have been found of distinct value in the detection of partial dehydration and latent edema.

The author employs the following method for the test as follows:

The patient urinates at exactly 7 A.M. and is given immediately 1000 cc of fluid either water alone or part tea and part water as he prefers. This amount of fluid is all to be taken within five or ten minutes. At 7:30 or 8 A.M. he may have rolls, toast and an egg or any other dry food. At 8 A.M. exactly the urine is to be voided and again at 9 A.M. Thereafter urine is collected every two hours until 9 P.M. The urine voided between 9 P.M. and 7 A.M. the following day is collected in one portion. Relatively dry food is given at lunch and supper usually potatoes, cheese, meat, eggs and toast. The patient is allowed to choose which of these he desires. If thirst becomes annoying a measured amount of water is given at 5 P.M. Usually however in the case reported no fluid was taken until 7 P.M. or 9 P.M. If the reaction on the part of the kidneys is normal to this test a diluted urine is voided during the morning hours and a concentrated urine during the remainder of the day.

TUBERCLE BACILLUS A Comparison of the Schultze-Tiggs and Ziehl-Neelsen Methods for Staining Acid Fast Bacteria, Simmons J S and Steves, E J Am Rev Tuberc July, 1926 xiv, 102

The authors conclude that both methods are of equal value.

LIVER FUNCTION The Rose Bengal Test for Liver Function with Particular Reference to Its Use in the Therapy of Syphilis Epstein A N and Rauschkolb J E Arch Dermat and Syph, August 1926, xiv No 2 p 122

With the usual preparation for intravenous medication, a vein in the cubital fossa is selected and a medium sized needle inserted. Ten cc of blood is withdrawn and transferred to a centrifuge tube, marked No 1 containing 0.4 cc of 2 per cent potassium oxalate solution made up in physiologic sodium chloride, which is inverted once gently. Rough handling may cause some hemolysis of the blood. The patient and blood specimens are kept in a subdued light to prevent hemolysis as a result of the photodynamic action of the dye.

The needle is left in situ and 10 cc of a 1 per cent solution of rose bengal (100 mg) is injected slowly. When 5 cc has been injected, the time is noted and the injection completed. The syringe with which the injection is made is discarded and a clean syringe containing from 3 to 4 cc of sterile physiologic sodium chloride is attached to the needle in the vein. The needle is kept free from blood clot by frequent injections of small amounts of sodium chloride solution. At the end of one and one-half minutes, blood is withdrawn slowly, so that when two minutes have passed the syringe contains 5 cc of blood. The blood is then transferred to centrifuge tube No 2 containing 0.2 cc of the potassium oxalate solution and the tube is inverted once gently. The syringe is washed with physiologic sodium chloride, refilled with the saline solution and again attached to the needle in the vein. In a similar manner an eight minute specimen is obtained and transferred to centrifuge tube No 3, containing 0.2 cc of the potassium oxalate solution.

This procedure differs from the original technique in that a four minute specimen is not taken, and that 0.2 cc of potassium oxalate solution, 2 per cent made up in physiologic

sodium chloride instead of 2 cc of 2 per cent potassium oxalate solution is used. This has the obvious advantage of requiring less manipulation of the vein, of necessitating less laboratory work and of giving as much information.

The blood specimens Nos 1, 2, and 3, control, two minute and eight minute, respectively, are centrifuged until the supernatant plasma is clear. The color in tubes Nos 2 and 3 is then compared with a standard solution of the dye made up as follows:

Five cc of the plasma in tube No 1 (control specimen) is added to 5 cc of a 0.0075 per cent solution of rose bengal, made up by adding 0.75 cc of a 1 per cent solution of rose bengal to 100 cc of water. This is thoroughly mixed, and 5 cc of the resultant solution is added to 15 cc of water. The original 5 cc of the 0.0075 per cent solution of rose bengal is thus diluted three times.

The plasma in tubes Nos 2 and 3 is diluted twice by pipetting 1 cc of plasma and adding it to 3 cc of water. These dilutions are used for convenience in colorimetric determinations. The diluted plasmas are then compared with the diluted standard solution in a Hellge colorimeter. Since the plasma of tubes Nos 2 and 3 are diluted in the same manner, the relationship between the color of each specimen will be constant.

Calculation of Results—The method of calculation, as originally described, required that the blood volume of each patient be calculated so that the colorimetric readings of the various patients might be compared with a standard. The arbitrary standard selected was an individual with a blood volume of 7000 cc who had received 100 mg of the dye, giving a resultant solution of 19.1 per cent of the standard prepared as above. The colorimetric reading of the plasma of the two minute specimen, after being corrected for dilution by the oxalate solution and for the solid elements of the blood which contained no dye, was reduced to 19.1 per cent at the expense of the blood volume. In other words, the colorimetric reading of specimen No 2 was reduced to 19.1 per cent. The eight minute colorimetric reading is then compared with the standard (19.1 per cent) by the following fraction:

$$\frac{\text{8 minute reading}}{\text{2 minute reading}} \times 19.1 \text{ per cent} = 19.1 \text{ per cent}$$

For example

The two minute colorimetric reading is 70 and the eight minute colorimetric reading 35, then to reduce this to standard conditions calculate as follows:

$$35/70 \text{ or } \frac{1}{2} \times 19.1 \text{ per cent} = 9.5 \text{ per cent}$$

Since 19.1 per cent is the highest concentration of the dye possible under standard conditions, this figure is 100 per cent retention of the dye in the blood stream. Since we are interested only in how much dye the liver can excrete in eight minutes, the eight minute reading, reduced to the standard conditions will determine whether a patient has a normal or abnormal liver function.

The upper limit of normal as previously established is 11.5 per cent for the eight minute colorimetric reading. Regarding 19.1 per cent as 100 per cent retention of the dye, the upper limit of normal will be 60 per cent retention, and we will consider an eight minute reading of more than 60 per cent as abnormal and denoting impaired liver function.

This report is based on a total of 252 tests applied to various types of syphilitic patients.

Latent hepatic disease occurring in patients in the various stages of syphilis can be detected by means of the rose bengal liver function test.

The authors frequently found the rose bengal liver function test a valuable indicator as absolute indication of liver insufficiency.

Arsphenamine jaundice is constantly associated with definite retention of rose bengal in the blood stream. With clinical recovery, the liver function returns to normal. There is no permanent change in liver function following chemical recovery from an arsphenamine hepatitis.

Acute syphilitic hepatitis probably sometimes occurs during the secondary stage without physical signs pointing to liver disease.

The authors frequently found the rose bengal liver function test a valuable indicator as to the prognosis in cases of arsenhenamino icterus. Moreover they believe it reveals cases of acute syphilitic hepatitis which cannot be diagnosed in any other manner. The test is an adjunct in the treatment of syphilitic patients.

PEROXIDASE A New Peroxidase Reaction Sato A and Sekiya S Tohoku Jour Ex per Med 1926, vii, No 5, p 111
Reagents

1 Copper sulphate 5 per cent aqueous solution.

2 Rnh 0.2 gm benzidine with a few drops of water in a mortar add 200 cc of water filter, and add 4 drops of 3 per cent hydrogen peroxide to the filtrate

3 1 per cent aqueous sanfranine

Method

To a fresh, dry blood smear add solution 1 drain, and add solution 2 for two minutes Wash in water

Place one drop of 0.05 per cent aqueous sanfranine on a slide and invert upon it the peroxidase-stained smear on a cover glass and examine microscopically

The peroxidase granules are stained a deep bluish green

CEREBROSPINAL FLUID The Mastic Whole Albumin Quotient as an Expression of the Albumin Proportions in the Cerebrospinal Fluid G Wullenwrbber Munchen med Wchnschr May 7 1926, lxxvii 772

The mastic solution is prepared according to Jakobsthal and Kafka it must be occasionally controlled by a preliminary test which is done, according to Lensberg's suggestion, by adapting the alkali content of a stable 0.8 per cent salt solution to the sensitiveness of the labile mastic solution. One to two drops of normal per 1 cc have usually been sufficient

One cc of the mastic solution for use is put on one of the 2 test tubes of Dold's flocculation meter together with 0.5 cc. of fluid and 0.5 cc. of a 0.8 per cent salt solution. One cc. of the mastic solution for use and 1 cc. of water are put into the other tube. The latter solution serves as a control. From the tube which contains the fluid and which shows more flocculation so much liquid is drawn into a measure tube that both tubes are equally flocculated. The degree of flocculation is determined and expressed in c.c. or 0.001 c.c. The changes of the fluid mastic mixture which, for instance in paralytic, lead to flocculation, are also read in this way as flocculation degrees. The determination may be made three minutes after the preparation of the reaction, always after the same time because the flocculation continues to increase. It expresses numerically the degree of flocculation, which in the mastic reaction is estimated after twelve to twenty-four hours.

The determination of whole albumin has been made by the author according to Mestre's diaphanometric method. The results are very exact they express differences of 0.01 per cent. In order to find the quotient, the flocculation degree of the mastic fluid mixture in c.c. is taken as numerator and the whole albumin content expressed in 0.01 per cent as denominator.

A table shows the result to the following effect

Meningitis fluid with little flocculation of the colloid solution and relatively high whole albumin content gives a small quotient below 1. Paralytic fluid with strong flocculation and lower whole albumin content, gives a big quotient, above 1. The highest values for menin-

gitis were $\frac{55}{0.9}$ and for paralysis $\frac{18}{0.6}$. Between the two extremities the numerical values lie exactly like the curves in colloid reactions. One case of cerebrospinal lues gave a transitional value, $\frac{1.2}{1.0}$ where numerator and denominator were nearly equivalent.

Besides distinguishing the different fluids of organic nervous diseases, accurately as the colloid reactions, the author's method offers several advantages. It substitutes the estimation of the whole albumin content numerical determination. The influence of the fluid on

sodium chloride instead of 2 cc of 2 per cent potassium oxalate solution is used. This has the obvious advantage of requiring less manipulation of the vein, of necessitating less laboratory work and of giving as much information.

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Acute syphilitic hepatitis probably sometimes occurs during the secondary stage without physical signs pointing to liver disease.

Rabbit hemoglobin (made by taking 1 part of defibrinated blood with 3 parts of distilled water)-----10 20 parts

2 Same containing fresh tissue

3 *Leptospira* medium containing glucose, maltose, inulin

4 Same, plus fresh tissue

5 Horse blood agar slant

Defibrinated horse blood added to melted 2 per cent nutrient agar (P_H 7.4) to give concentration of 20 per cent

6 Same, containing glucose maltose, inulin

7 Plain agar slant

8 Plain broth

The strain of *Bartonella bacilliformis* thus isolated grows well on the semisolid *leptospira* medium, and also on slant agar containing animal blood. The initial growth is not readily recognizable to the naked eye, but the presence of the organisms can be determined by means of the dark field microscope and by Giemsa and Gram staining methods. No growth has been obtained on the more ordinary culture media. The organism is an obligate aerobe, is Gram negative, and under certain cultural conditions motile. All the forms which have been described as occurring in human red corpuscles may be found in the cultures and in addition many granular and coarsely irregular forms have been met with.

The inoculation of cultures of *Bartonella bacilliformis* into *Macacus rhesus* produce infection and gives rise to effects which differ with the mode of inoculation. The intravenous injection of the culture into young macaques induces a prolonged irregularly remittent fever. The organism can be cultivated from the blood over a long period, and it has been detected within the red corpuscles of the monkeys, reproducing the precise appearance observed in human cases of Oroya fever.

The intradermal injection of the culture into the eyebrow of young macaques gives rise to nodular formations rich in new blood vessels and showing the bacilliform organism within the endothelial cells. From the experimentally induced nodules cultures of the organism are readily recovered.

The paper contains fifteen microphotographs and two colored plates.

PREGNANCY SERUM DIAGNOSIS The Ninhydrin Flocculation Test a New Reaction in the Blood Serum for Determination of Pregnancy Vogel W Zentralbl f Gynak June 12, 1926, 1, 1554

Place 175 cc of blood serum 10 cc of water (see below) and 0.2 cc of 1 per cent ninhydrin solution in a test tube and boil three minutes in a water bath.

The albumin of nonpregnant serum forms coarse flakes which readily separate from the bluish opaque fluid. In serum from pregnant women the albumin either remains in solution or if the pregnancy is early fine bluish flaky precipitation occurs.

Upon standing in positive reactions a light finely flaked grayish blue or blue precipitate covers the bottom of the tube and is readily resuspended on shaking.

With a negative reaction the flakes are coarse, fill about one third of the tube and frequently turn a reddish violet.

It is necessary to use a specially prepared 'standard water. Two and five tenths grams of bicarbonate of potassium are dissolved in 94.5 cc of distilled water to which are added 55 cc of official lime water solution. The air is forced from a 10 liter flask by admission of carbonic acid from an ordinary carbonic acid bomb tube with the pressure regulating valve.

The solution obtained is put into the flask filled with carbonic acid gas, the opening closed with the palm of the hand and the flask is shaken until the hand is no longer sucked into the opening. The finished solution is kept in seltzer water containers with patent stoppers.

Such a standard solution is stable only for 6 to 8 days and is useless when it becomes transparent during the test.

The blood must be taken from the fasting patient.

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REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building,
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*Textbook of Bacteriology**

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The first section of the book (seven chapters 171 pages) is devoted to an excellently presented exposition of various technical procedures.

Section II (607 pages) is devoted to systematic bacteriology concerned especially with bacteria responsible for disease in man and animals. This section is exceedingly well done, its contents are readily accessible and of immediate practical value and it is one of the most easily utilisable the reviewer has encountered.

In Section III (38 pages) the distribution of bacteria is discussed followed by 96 pages devoted to infection and immunity in Section IV, also a very excellent presentation.

The fifth section (68 pages) is devoted to spirochetes, and the last section (54 pages) to the various agents of undetermined character.

The illustrations, free hand drawings are excellent in every way and are truly illustrative without being diagrammatic.

The book can be recommended without reserve as an authoritative comprehensive, and utilisable text.

Recent Advances in Hematology†

AS STATED by the author in his preface, 'the production of an adequate book dealing with recent advances in hematology would require an almost encyclopedic knowledge of the vast literature of the subject, but while the present volume is small and compact, it represents an admirable survey of a complicated and most important subject.

Cytologic blood examinations, with which this book is entirely concerned are perhaps one of the most frequently applied of clinical laboratory procedures.

Like all laboratory methods of examination they achieve their greatest clinical value when properly interpreted, and to this end the matter clearly and excellently presented by Dr Pinoy brings much that is not only of interest but of marked clinical value.

The first two chapters are devoted to a consideration of the reticuloendothelial system and the development of blood cells.

Pinoy says "The fact that the formed elements of the blood are not developed in the circulation, but are derived from the activities of parent cells in various organs makes clear the error of the conception of the blood as a special form of connective tissue with a fluid matrix. It is really in the nature of a mixed secretion of a variety of origins.

The review of the reticuloendothelial system is an excellent exposition of a disputed subject.

Textbook of Bacteriology By William W Ford M.D. Professor of Bacteriology School of Hygiene and Public Health Lecturer on Hygiene School of Medicine Johns Hopkins University. Octavo of 1069 pages with 186 illustrations. Cloth W B Saunders Co Philadelphia and London 1927.

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The typography and illustrations which are well chosen and reproduced, constitute an excellent example of the printer's art

The volume can be unhesitatingly recommended to the student and practitioner and will be of use in the laboratory as a reference volume

*Differential Diagnosis of Internal Medicine**

HEALTH and disease may be looked upon as opposite extremes of functional efficiency health being regarded as a condition dependent upon perfect performance of function and disease a condition characterized by disturbance or loss of function

Diagnosis, the first essential step in the management of disease rests upon the determination first of the particular function or functions affected second of the degree of impairment third, whenever possible upon the detection of the responsible cause and its mechanism

In the general practice of medicine the presenting the obvious or the troublesome symptom is as a rule, the direct cause of the visit to the physician and unless carefully considered and correlated with other findings may often overshadow the more important underlying cause

It is as an aid in such correlation as a stimulus to further clinical studies and is an indicator of diagnostic possibilities that such volumes as that of Professor Mathes find their true use

In this the translation of this well known German text the translators have included American and English methods applicable to the differential diagnosis of disease and have made such other additions as were required to bring the work up to date

The twenty four chapters of this work include a consideration of the differential diagnosis of acute febrile conditions diseases characterized by moderate fever the meningeal and peritonitic symptom complexes ileus and intestinal stenoses diseases of the larynx and trachea diseases of the lungs and pleura circulatory diseases the spleen, liver, and biliary ducts the gastrointestinal tract the pancreas the urinary organs the blood endocrine glands diseases of bone neuralgia like pains and headache

The discussion under these headings is clearly written in an eminently practical manner which emphasizes the practical experience of both the author and the translators

Diagnosis is an art to be learned by studies of the patient and cannot be acquired from books alone Books, however are valuable aids in such studies as crystallizing the impressions formed and there are few volumes of this type that will prove more generally useful to the physician at large to whose library Professor Mathes' book will be a valuable addition

Typographically the volume is an excellent example of the printer's art

City Health Administration†

THIS is a very good outline of the principal functions involved in municipal health administration, of which the author has made a study of interest not only to the medical man and the sanitarian but in which he has also tried to keep the layman's viewpoint in mind The author has approached the subject matter from the viewpoint of a student of municipal government as a whole, and it is not surprising that his recommendations embody a rather close association or amalgamation between public welfare and public health An exception to this viewpoint may be taken by the health administrator who has been closely identified with the endeavor on the part of progressive health departments to unload the driftwood of charity and welfare and other activities having only an indirect influence upon prevalence of disease

* *Textbook of the Differential Diagnosis of Internal Medicine* By M. Mathes M.D. Professor of Medicine University of Königsberg. Authorized translation of the fourth German edition by Drs. I. W. Held and M. H. Grob. Pp. 908. 16 illustrations. Cloth. P. Blakiston & Sons and Co. Philadelphia

† *City Health Administration* By Carl E. McCombs M.D. National Institute of Public Administration and New York Bureau of Municipal Research. Pp. 24. Cloth. The Macmillan Company, New York 1927

Dr McCombs has not drawn to any great extent upon the recommendations which have emanated from the various Committees of the American Public Health Association, and more especially, the Committee which has been responsible for the formulation of the Appraisal Form for City Health Work. On the other hand he shows that he has a good acquaintance with the purposes of the Association and its ideas and ideals which are representative of the best thought among the American health administrators.

His book is divided into three parts, the first dealing with general municipal health functions. It is the second part which will prove of the greatest interest to the sanitarian, and this section constitutes by far the greatest portion of the book. The author discusses the organization and administration of sickness preventive functions, taking up in proper sequence the organization of a board of health, a description of the duties and responsibilities of the health officer and his employees, an outline of a vital statistical service and bureaus for the prevention and control of disease, promotion of child hygiene and public health nursing, food inspection, sanitary inspection, laboratory service, and public health education. The third portion of the book is devoted to a discussion of the organization and administration of sickness treatment functions with chapters on hospital management, medical and nursing services of hospitals, record keeping, etc.

The book is written in an orderly fashion, and each discussion leads most appropriately to the paragraphs that follow. The general outline is admirable, although it must be admitted that in some of the detailed recommendations there could not be complete approval on the part of those who have had many years of practical experience in municipal health administration. The health administrator has waited for many years for a good outline on administrative procedure, and it is the opinion of the reviewer that to date, at least, the present volume is the best and most modern contribution which has come to his attention.

*Ergebnisse der medizinischen Strahlenforschung (Roentgendiagnostik, Roentgen-, Radium-, und Lichttherapie)**

THIS is the first of a series of volumes containing monographs written by those eminent in various fields of radiology. Although the number of volumes to be issued is not stated, it is intended that both clinically and theoretically all phases of radiology will be adequately covered. There are ten monographs in this volume which consists of approximately 700 pages, printed on good quality paper with adequate illustrations of high quality. Abundant clinical data is given to support the conclusions drawn, and the methods employed are fully explained. The monographs of clinical interest include *Inflammation and Malignant Conditions of the Large Intestine* by Fischer, *Bone Atrophy* by Friedl and Schinz, *Acute Miliary Tuberculosis* by Lorey, *The Treatment of Carcinoma of the Cervix* by Lahm, and *X Ray and Radium Treatment of Carcinoma of the Esophagus* by Kurtzahn. The general and theoretical subjects treated are *The Spectroscope in Medical Roentgenology* by Grebe, *The Ionization Measurement of X ray* by Kustner, *The Effect of X ray on the Testicle* by Schinz and Slotopolsky, *Physical Sensitization* by Holthuse and *Protection Against X-ray and Plans for a Roentgen Division of a Hospital* by Glocker.

In his *Treatment of Carcinoma of the Cervix* Dr Lahm reviews the end results of operation in carcinoma of the cervix, and gives an extensive comparison of treatment by operation and by radiation. Five years freedom from recurrence is considered a cure, although it is recognized that in rare instances recurrences occur after ten to fifteen years. The results of Doderlein and Kroenig are among those extensively quoted. In general, approximately 20 per cent of the cases remain symptom free for five years when treated surgically, and the same percentage, when treated by radiation. Radiation of inoperable cancer gave approximately 10 per cent cure in a series of 2,000 cases from seventeen different clinics. The author concludes that operation and radiation when in competent hands are of approximately equal value.

*Ergebnisse der medizinischen Strahlenforschung (Roentgendiagnostik Röntgen Radium und Lichttherapie) Edited by H. Holfelder. Frankfurt a. M. H. Holthusen. Hamburg. O. Jungling. Tübingen. H. Martius. Bonn. a. Rh. Vol. I. Published by Verlag von Georg Thieme Leipzig 1925.

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EDITORIALS

The Cardiac Minute Output or the Velocity of Blood Circulation

WITHIN the last year considerable interest has been manifested in the velocity of blood flow, the circulatory minute volume in the peripheral arteries, and also in the pulmonary and the coronary arteries, the cardiac output, the pulse wave velocity and the capillary bed reaction, all under normal and abnormal conditions. Many ingenious methods have been evolved for the determination of each of these rates and the establishment of some recent conceptions of the mechanism of the circulation and some of the controlling factors. The volume of carbon dioxide produced to the volume of oxygen consumed and the difference between the arterial and the venous oxygen tensions are indices of the cardiorespiratory function. The newer methods for obtaining these facts in man are most promising. The results have already led to rather striking conclusions.

The original method of Fick has been much elaborated and perfected by Burwell and Robinson (1*) and has been extensively employed by them with human subjects and by Harrison, Leonard, and Blalock with dogs in the

The roman numerals indicate the number of the respective study in the series of studies by the group of authors whose work is being discussed

accumulation of much interesting data. Robinson and his coworkers consider the evidence secured as satisfactorily establishing the facts that (II) the output of the heart is greatly increased when heart failure sets in and reduction of the output of the heart is favorable to the reestablishment of adequate functional circulatory efficiency. (III) Digitalization, it was demonstrated in animals and later in man, produces, contrary to expectation, a fall in cardiac output.

This group of workers has by this method further demonstrated that (IV) the cardiac output in pneumonia is usually increased, depending not on changes in oxygen consumption, but on the accompanying anoxemia and the anemia. The strain on the myocardium is thus increased. (V) Diminution of the hemoglobin content of the blood is associated with an increased cardiac output. Progressive hemorrhage, on the other hand, leading to decreased blood volume, causes no marked change in the cardiac output until the animal approaches a state of shock when the cardiac output rapidly decreases. With the maintenance of the blood volume by saline, with, nevertheless, an unavoidable diminution of the hemoglobin by dilution, there is an increased output which diminishes with shock. (VI) Anoxemia of severe degree (30 per cent unsaturation) causes increases up to 500 per cent in the minute cardiac output.

Besides the effects of digitals (I) on the cardiac output of dogs, Pilcher, Wilson, and Harrison have studied the effects of barium, calcium and potassium (II), caffeine sodium benzoate (III), and epinephrin (IV) on the circulatory minute volume in dogs. In these studies, some unexpected results were again obtained. For instance, caffeine sodium benzoate in full therapeutic doses showed only slight, but definite, decrease in the minute ventricular ejection. Epinephrin, however, as was to be expected, produced, as an immediate effect, an increase in the output of the normal dog's heart. Thus, the treatment of "shock" or hemorrhage which had previously been proved to decrease the cardiac output was, according to the authors, rationalized.

It is fortunate indeed that paralleling these studies of the Nashville group, there are, in almost equal number, studies of similar phenomena by a new and altogether different means of approach.

Blumgart, Weiss, and Yens have perfected (I) an active radium deposit intravenous injection method for the determination of the velocity of blood flow. The estimation of the circulation time from the injection into one arm to the appearance of the radium in the opposite arm shows less than a three second variation. (II) The average arm to arm circulation time for normal individuals of all ages was eighteen seconds, when reduced to square meter of body surface it was ten seconds at the ages of fifteen to twenty nine, twenty seconds between thirty and seventy-five years. The velocity of blood flow is more rapid in children. Increased pulse rate increases the velocity of blood flow in one and the same individual. Normal low ventricular rate, however, does not lower the velocity below that for other normal individuals with higher rates. Normal variations in blood pressure have apparently no great effect on velocity of blood flow. (III) In rheumatic and syphilitic heart disease (A) without valvular damage, three patients with cardiac over-

activity of rheumatic origin showed normal or slightly increased velocity. Three with signs and symptoms of severe myocardial damage showed slightly prolonged velocity. (B) With valvular lesions and regular rhythm, eight studies showed circulation times within normal limits in the absence of cardiac decompensation and with slight or great prolongation according to the degree of heart failure. The venous pressures were normal. The vital capacities were reduced. The dysfunction thus, is seemingly due to myocardial damage. The site and extent of the valvular lesion seemed to play no part. (C) With valvular disease and fibrillation of the auricles (I) the circulation time was twenty nine to fifty five seconds, depending upon the circulatory compensation.

In syphilitic heart disease with aortic valve lesions the circulatory velocity was prolonged only where there was evidence of circulatory failure and in degree corresponding with cardiac pain and aortic dyspnea and with no signs of congestive failure normal velocity of blood flow may be present. This suggests that these symptoms are not necessarily due to congestion of the pulmonary vessels, but may be due to a reflex mechanism. In 53 normal individuals the range of blood flow velocity was from eleven to twenty four seconds, averaging eighteen seconds while in 86 cardiovascular cases it was from eleven to seventy three seconds, averaging thirty three seconds.

(IV) In arteriosclerotic cases without symptoms and signs of cardiac failure, the average was twenty four seconds while in the same type of case with symptoms and signs of cardiac failure the average was thirty eight seconds. Thus a slight retardation in velocity occurs in general before symptoms and signs of cardiac failure become manifest. The degree of retardation corresponds to the degree of cardiac failure.

In *hypertension* cases *prolongation* of the velocity of blood flow was observed without the presence of any evidence of circulatory embarrassment. Auricular fibrillation complicating any case induces a disproportionate prolongation over those with regular mechanism and cardiac failure. Vital capacity reduction appears first then velocity retardation then venous pressure increase. With recovery from failure venous pressure first returns to normal then velocity of blood flow, and lastly vital capacity.

Retardation of velocity appears before symptoms and signs of failure and returns toward normal before there is clinical evidence of improvement.

(V) The physiologic and pathologic significance of the findings in regard to velocity of blood flow are reiterated. The path traversed by radium C is considered proved to be uniform from patient to patient. Excluding all possible local causes, the authors have found that the rise in venous pressure is preceded by a definite period, when the vital capacity is reduced and the velocity of the blood flow is lessened. A study of the anatomic and physiologic characteristics of the veins is thought to afford the explanation of this precedence.

The authors then elaborated their radium active deposit method so as to detect the ionization current produced by the gamma rays at the moment that the radium active deposit reaches the right heart as well as detection of the time of arrival in the arteries about the elbow. The difference between

these times after the application of a standard correction gives a measurement of the velocity of blood flow through the lungs. In 50 normal individuals, the pulmonary circulation time ranged from four and a half to seven teen seconds, with an average of eleven seconds. In cardiac failure the pulmonary circulation time was sixty-eight seconds, while the venous circulation time was thirty-four seconds. In emphysema, the velocity of blood flow through the lungs was found to be normal. Digitalization of normal individuals produced no demonstrable change in the velocity of pulmonary blood flow, whereas, in cardiac patients in whom there was definite clinical improvement following digitalization, the velocity of blood flow through the lungs was increased.

This finding of increased velocity of pulmonary blood flow following digitalization apparently coincides with the clinical conception of the effect of digitalis and seemingly disagrees with the unexpected conclusions of Robinson, Buiwell, and then coworkers. It may be, however, that the effect of digitalization may be distinctly different on the pulmonary circulation as contrasted with the systemic circulation. Vascular changes resulting in pressure changes have been shown by both methods to produce changes in the cardiac output, and it may possibly be that the output of one or the other ventricle can be independently affected.

Blocklehurst, Haggard, and Henderson estimated that about 100 cc of blood is pumped on through the lungs and on to the tissues for each 4 cc of oxygen consumed at rest. The circulation efficiency is so great that the arteriovenous carbon dioxide difference is on the average only about 3.5 per cent by volume and the oxygen difference only slightly above 4 per cent by volume. An oxygen consumption of 240 cc per minute requires a circulation of 6,000 cc of blood. The normal circulation is thus such that with the blood giving up only 4 per cent by volume in passing through the capillaries, the tissues are kept in a pressure of oxygen. The efficiency of the circulation depends on its capacity to maintain these conditions. The measurable factor of this limitation of power is the relation of the oxygen content of the arteries to that of the venous blood. With inefficiency, the difference increases. This is what is termed an increased oxygen debt.

Henderson, Haggard, and Dolley have studied men of various grades of activity and observed that in passing from rest to exercise, the nonathlete approximately doubles his circulation, while the athlete triples his blood flow. In moderate exertion, the adjustment is proportionate to the oxygen consumption and the energy expenditure. The average normal stroke volume at rest is about 1.5 to 1.8 cc per kilogram of body weight per beat. In the athlete, the pulse rate tends to be slower and the stroke volume distinctly greater at rest as well as on exertion by which it may be increased 50 per cent. Increasing pulse rate decreases the stroke volume. The slow pulse allows longer diastoles for better relaxation and filling of the ventricles.

Halsey, Reynolds, and Blackberg, of Tulane University, using a slightly modified technic of Marshall, made some important observations. These investigators found the cardiac output in the dog usually increased by chloral hydrate, quinine, and quinidine, and decreased by chloroform, homocamphor

(eyelosal) and ephedrin alone or after chloral and chloroform. The accompanying heart rate, blood pressure and oxygen consumption rate changes were recorded and are carefully discussed as are, also, the possible explanations of the findings. More of this type of fundamental and laborious pharmacologic research will guide clear and rational conceptions of the effects of drugs.

However there is as yet not sufficient corroborated data obtained by various methods and various groups of workers to justify sweeping conclusions or to substantiate new theories. New and interesting light has been shed on these phenomena. The improved ethyl iodide method of Henderson and Haggard, an improved method which J. W. Moore and his associates are working with and the promise of an improved method from E. H. Marshall, all together hold forth hope that the next few years will yield much corroborated data on the subject of the velocity of blood circulation and the minute cardiac output under normal physiologic, pharmacologic and pathologic conditions.

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—G R H

The Advantage of Early Examination of Diseased Tissue

WHEN a patient presents himself with a lesion of the skin, particularly of the face, which is suspected of being cancerous, the most important point to determine is the type of the lesion. Owing to propaganda and the general education of the public with regard to cancer, physicians generally are seeing malignant lesions earlier than formerly. A fairly certain method of determining the type of lesion is either by biopsy or by excision of the diseased area and its examination with a microscope. The local lesion itself is not the most important consideration, for by known therapeutic agents (excision, electric coagulation, or irradiation) this area can usually be controlled. Of much more importance is the determination of the type and grade of malignancy of the original tumor, if it is a neoplasm, so that intelligent procedures regarding the adjacent lymph nodes can be planned and executed.

Certain observers have assumed that biopsy is dangerous because of the risk of causing more rapid spread of the growth. The evidence usually offered was the attending physician's impression that growth is more rapid following removal of tissue. On the other hand, experimental evidence has accumulated in carefully controlled observations to support the opinion that biopsy of a malignant tumor has no apparent influence on its growth or spread.*

It seems more logical to run the small risk, if there is such, in biopsy for the purpose of examining excised tissue and establishing a definite diagnosis, rather than inaugurate treatment for a lesion whose exact nature has not been determined.

It frequently happens that an early lesion of the skin is treated and destroyed, and the site of the affection heals without the exact pathologic change having been determined. Later adjacent lymph nodes may enlarge and subsequent examination of tissue reveal carcinoma. If the original lesion or a part of it had been examined microscopically, the type and grade of malignancy determined, and appropriate treatment instituted, such as the removal of adjacent lymph nodes, the patient might have been given a better prognosis.

If chronic lesions develop in affections of the skin, particularly of the face, in which malignancy must be considered in the differential diagnosis, much valuable time is saved if a part of the diseased area which is a fair sample of the whole, or the entire lesion, is excised and examined by a pathologist.

—H D C

*Wood F C The Experimental Pathology of Cancer *Jour Am Med Assn* 1925 lxxxiv 4-8

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Annual Meeting of the American Society of Clinical Pathologists

The next annual meeting of the American Society of Clinical Pathologists will be held in Minneapolis, Minnesota June 8 9 and 11, 1928 headquarters being at the Hotel Leamington

Members are urgently requested to send in their reservations to the Secretary at once if they have not already done so

The next meeting will be an extremely interesting one judging from the titles of the papers that have been sent to the Secretary Below is a provisional list of the titles submitted up to date

Preliminary Program

TENTATIVE

- Pertinent Facts Concerning Hemoglobin By C E Poderick M D, Battle Creek, Michigan
- Blood Transfusion Studies By Harold G Palmer M D Philadelphia, Pennsylvania
- The General Practitioner and the Early Diagnosis of Cancer By Wm C MacCarty, M D Rochester, Minnesota
- Further Observations With a New Method for Cultivating Tubercle Bacilli A Comparison With Guinea Pig Inoculation and Petroff's Method By H J Corper, M D, and Nao Uyei, Ph D, Denver, Colorado
- The Specificity of Bacteria to the Bacteriolytic Action of Chemicals With a Note on This Application to Chemotherapy By Robert A Keilty M D, Washington D C
- B Abortus, a Clinical and Bacteriologic Study By A S Giordano M D South Bend Indiana
- The Sedimentation Time of the Blood in Jaundice By Nathan Rosenthal M D New York City
- Value of Nuclear Deviation (Arneith Schilling Formula) in Blood Examinations for Clinical Medicine By F W Nicholas M D, Omaha, Nebraska
- The Chemistry and Cytology of Serous Fluids By A G Foord M D, Buffalo New York
- A Report on Our Work on Epilepsy By A P Saunders Chicago Illinois
- Complement Preservation, a Study in Actual Practice By B W Rhamer M D Fort Wayne Indiana

The oral administration of pollen was practically coseasonal. Since it was learned that protecting doses could be reached quickly, it was unnecessary to begin treatment weeks before the season. Patients who presented themselves before the onset of the season were told to report for treatment on appearance of the first symptoms. Those appearing after the development of symptoms were started on treatment at once. The extract was dispensed in dropper-stoppered bottles. The initial dose was 10 drops of a 1:20 extract. Each succeeding dose was increased by 10 drops until 60 drops were being taken at a dose. Doses were taken three times daily so that the dose of 60 drops was reached within forty-eight hours. The extract was dropped into a glassful of water, milk, or other beverage, stirred well and drunk. The usual instruction was to take it before meals, but occasional individuals found some nausea occurring when the stomach was empty, which could be prevented by taking the pollen after meals. I was unable to find any difference in the results dependent upon the presence or absence of food in the digestive tract. The dose of 60 drops was made arbitrarily the maximum after I had found that patients who did not show any protection at that dose did not do any better if the dosage was carried higher. Under other conditions, such as different pollen environment and the use of different extracts, the dosage required to protect might be entirely different. That there is a maximum dosage, beyond which it is useless to go, seems apparent. We found no appreciable difference in the dosage of children and adults. In those patients who secured protection by 60-drop doses, this dose was maintained for a few days and then lowered five drops per day until the minimum was reached as shown by the reappearance of symptoms. There was no uniformity in this respect. A few patients remained well on doses of 20 drops once a day. Others required more, and many required the 60-drop doses three times daily. The variation in this respect is quite similar to that which is found in the protecting dose of hypodermic treatment.

The cases reported are unselected and represent all the cases treated during the second half of 1927 except those whose treatment is still in progress, those who, for one reason or another, did not carry treatment far enough to determine results, and those from whom we have been unable to get reports. The percentage of improvement shown in Table I is that claimed by the patient, checked whenever possible by my own observation, and is believed to be a conservative statement. Under the heading "75 per cent" are grouped those patients who could claim 75 per cent or more of improvement but could not be listed as 100 per cent cases. Likewise, the 50 per cent group is made up of those whose percentage of improvement ranged from 50 per cent to 70 per cent. I believe this is a conservative method of presenting the figures and that, as presented, they do not overestimate the benefit obtained.

Table I presents the number of cases and the percentage of the total number of both hay fever and asthma patients obtaining the different degrees of improvement. The total number of hay fever patients treated orally (73) is slightly greater than the number receiving hypodermic therapy (61), but the number of asthma patients treated orally (18) is small compared with the number treated hypodermically (32). The difference in the number of asthma

TABLE I

RESULT	HAY FEVER				ASTHMA			
	ORAL		HYPODERMIC		ORAL		HYPODERMIC	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
100%	13	17.80	15	24.60	6	33.3	16	50.0
75%	25	34.25	33	54.10	6	33.3	8	25.0
50%	15	20.55	9	14.75	2	11.1	7	21.87
25%	5	6.8	0	0.0	0	0.0	1	3.15
0%	15	20.55	4	6.55	4	22.2	0	0.0

patients treated by the two methods was not due to the difference in the results expected but to the fact that pollen therapy by mouth over long periods of time is usually quite expensive.

It is apparent that the results with hypodermic therapy are better than those with pollen given orally. This is evidenced not only by the larger percentage who received substantial benefit from treatment but also by the smaller number who got no appreciable results from treatment. Of those obtaining 75 per cent or more of improvement—which I consider quite satisfactory—there is a definite difference in favor of hypodermic therapy. There were many more complete failures with oral treatment.

In 13 cases of hay fever I changed from oral to hypodermic treatment because of gastrointestinal symptoms or because they were getting too little improvement. Six cases of asthma were similarly changed. Three cases of hay fever and one of asthma were changed from hypodermic to oral therapy because of violent systemic reactions from the former method. The percentage of improvement in each type of treatment is shown in Table II. It is evident from these figures that a failure to get results by oral treatment does not preclude the possibility of controlling the case by hypodermic therapy and that some patients in whom severe reactions prevent satisfactory hypodermic treatment a change to oral administration may be of benefit.

TABLE II

CASES CHANGED FROM ONE METHOD TO THE OTHER

ORAL TO HYPODERMIC				HYPODERMIC TO ORAL			
HAY FEVER		ASTHMA		HAY FEVER		ASTHMA	
ORAL	HYPO	ORAL	HYPO	HYPO	ORAL	HYPO	ORAL
0	50	0	0	90	90	60	75
0	60	0	0	75	90		
0	75	0	75	75	90		
0	100	75	75				
25	75	75	75				
25	90	75	90				
25	90						
50	50						
50	80						
50	80						
75	75						
75	75						
75	90						

The figures represent the percentage of improvement received.

Six of the hay fever patients changed from oral to hypodermic therapy were changed because of nausea, diarrhea, and abdominal distress. There were a few others who had slight nausea, but these were relieved by changing

the time of dosage from before to after meals. In all these patients the abdominal symptoms came on within from twenty-four to forty-eight hours after oral treatment was begun and persisted until it was stopped. None of the usual medicinal agents gave any relief, but symptoms disappeared promptly on discontinuance of treatment. These patients were then put on hypodermic treatment with no recurrence of symptoms. The natural assumption, I think, is that these individuals have a sensitization of the mucosa of their digestive tract as well as of their respiratory tract, but I have no other evidence to substantiate such a belief. It is interesting to note—although it may have no significance—that all of these six patients were made ill by *ragweed* pollen. It is possible that these symptoms might be avoided by giving the pollen more frequently in smaller doses, but I have not yet done this.

In changing from the oral to the hypodermic method, an interesting and important finding was made. In those patients who had received a considerable degree of protection by oral treatment, it was possible to begin hypodermic treatment at a high dose, usually as much as 0.1 c.c. of a 1:20 dilution could be given safely. However, in those who got little or no protection from oral treatment, hypodermic treatment had to be started at the beginning as though they had had no treatment at all. Failure to recognize this fact caused some severe systemic reactions. These patients, after taking large quantities of pollen by mouth, were apparently as sensitive to it as formerly. In these cases I have been unable to find any of the "atopen" in the blood stream, and I believe that, for some unknown reason, there is little or no absorption of the pollen.

I have always had a great deal of difficulty in carrying up the hypodermic dosage of careless weed cases. Systemic reactions are frequent and severe in spite of all precautions, and carrying a patient to his "protecting dose" is a prolonged and difficult procedure. In the three cases reported in which careless weed was a factor, I have adopted the plan of giving the careless weed by mouth even though the other pollen to which the patient is sensitive may be given hypodermically. In these three cases protection has been secured with a very welcome absence of systemic reactions.

None of the patients receiving pollen orally had any asthma, hay fever, or urticaria as a result of treatment. Early in this work several patients received as much as 300 drops—equivalent to approximately 15 c.c.—of the 1:20 extract each day for three or four days. In none of them was there any evidence of symptoms, such as I see frequently from hypodermic therapy. In contrast to this six hay fever patients and one of asthma, treated hypodermically, had violent systemic reactions. Three of these patients refused further treatment because of fear of another reaction.

The oral administration of pollen has certain definite advantages over hypodermic therapy.

First, treatment does not require a prolonged preseasonal period but is entirely coseasonal. In this respect it is of distinct advantage in those patients who present themselves for treatment after the season has begun.

Second, patients do not have to report to the physician for each dose of pollen but can carry on the treatment under his direction at home. This saves

much time for both physician and patient, makes it possible to care for a much larger number of patients and permits the treatment of out of town patients and travelling men without the fear of reactions from improper or excessive dosage

Third, the extract diluted largely in water milk or other beverage is almost tasteless and children take it without objection. The pain, which is such an objectionable accompaniment of the injection of the glycerin saline extracts, is avoided

Fourth systemic reactions do not occur. This is in marked contrast to the rather frequent occurrence of reactions in those treated hypodermically. It is a source of much satisfaction to both patient and physician.

The disadvantages of oral treatment are

First the material in the amounts usually used is expensive. This becomes a matter of importance in prolonged administration and in the use of those pollens whose cost is high. I have not attempted to use this method in many cases of asthma because the continued use of large doses was too expensive for most patients. I have used it frequently to get the patient under control promptly and then changed to hypodermic dosage to maintain protection. This is an excellent time saving plan in those patients who respond to oral therapy. I have not attempted oral treatment with such pollens as mountain cedar because its cost is prohibitive. Timothy grass and the rag weed pollens may be bought in quantities at a low price and lend themselves well to this manner of use.

Second a small percentage of patients cannot take pollen by mouth because of nausea and abdominal pain. It is possible that they might be able to take it if the dosage were divided into small amounts and taken more frequently but I have not tried this method of usage.

Third, the percentage of those securing satisfactory protection is less than with hypodermic treatment and the percentage of complete failures is considerably higher. The figures might be somewhat different if the series were larger but I believe the conclusion stated is justified. If some method might be devised by which one could determine whether a given patient would respond to oral treatment without waiting for a clinical test at the onset of the season, it would be a most desirable thing. At present if oral treatment is begun at the onset of the season and fails to protect the patient, it makes it necessary to begin coseasonal hypodermic therapy the patient suffers unnecessarily and the results are not so good. At present there is no method available for eliciting this information.

SUMMARY

A comparison is made of results obtained by oral and by hypodermic pollen therapy. The conclusion seems justified that oral treatment has certain definite advantages but that satisfactory protection is secured less often

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PRACTICAL CONSIDERATIONS OF METAPLASIA IN NEOPLASTIC DISEASES*

BY HAROLD D CAYLOR, M D, ROCHESTER, MINN

THE term metaplasia may perhaps be given properly to the transition of one tissue into another of a related kind or to the formation of different tissues from a common parent cell. By certain observers metaplasia is defined as the production of tissue by cells that usually produce a different type of tissue. This change of one type of tissue to another has been seen in many organs, including the skin, gall bladder, stomach, and uterus. Practically, metaplasia has been of little general interest and importance. It has been well described by Lubarsch and others. The present observations concern meta

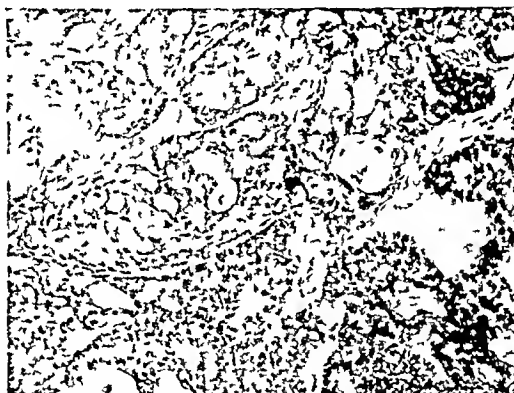


Fig 1—Adenocarcinoma of the breast (x120)

plasia of columnar epithelium to squamous epithelium, and its practical applications to the diagnosis and treatment of neoplasms.

Lubarsch,³ in referring to all changes of cell form in general, used the term "alloplasia" or "heteroplasia" and, under this heading, outlined three main groups: (1) pseudometaplasia, (2) metaplasia, and (3) undifferentiation. Pseudometaplasia he described as "a simple change of the form of cells, metaplasia as a genuine change of a specific cell or tissue to another of similar kind, and undifferentiation as the change of sharply differentiated cells into undifferentiated cells." The last group was divided into two types: physiologic undifferentiation by indirect nuclear division, which was the ordinary mode of cell division, and pathologic undifferentiation which was called by von Hansemann "anaplasia" and by Beneke "kataplasia." Present interest is in the second group, metaplasia.

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EXAMPLES OF METAPLASIA

Squamous cell epithelioma developing in the gall bladder is an excellent example of metaplasia.² Normally there is no squamous epithelium in the gall bladder or in any of the adjacent organs, and it is by assuming that this tumor arose partly because of the change of the tissue by metaplasia that its origin can be explained. Another example of metaplasia in epithelial tumors is a squamous cell epithelioma developing in malignant papillary cystadenoma of the ovary.⁴ A diagnosis of metaplasia in a neoplasm can be safely made only



Fig. 2—Metastatic adenocarcinoma in an axillary lymph node. (x10)



Fig. 3—Squamous cell epithelioma in an axillary lymph node. (x10)

after careful examination of the body has failed to reveal another tumor. Two different but related neoplastic disorders can be present in the same portion of the body at the same time. Two kinds of metastatic tumors may occur in lymph nodes from two primary malignant neoplasms elsewhere in the organism. To determine whether two related masses present in lymph nodes are due to multiple lesions elsewhere, or to metaplasia of one type of tumor to another, careful search must be made for distant primary lesions.

I have observed areas of metastatic squamous cell epithelioma in the axillary lymph nodes in cases of adenocarcinoma of the breast. The growth in the breast and in most of the involved lymph nodes was typical adeno-



Fig 4—Cervical polyp containing areas of squamous epithelium (x60)

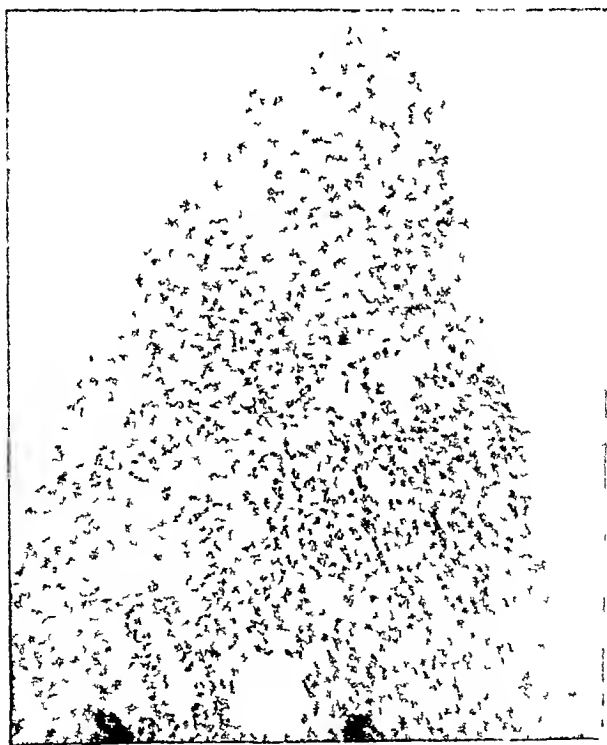


Fig 5—Portion of a cervical polyp covered in part by squamous epithelium and in part by columnar epithelium (x200)

carcinoma (Figs 1, 2, and 3) Careful examination of the excised tissue did not reveal a primary epithelioma and evidence of the presence of any other neoplasm could not be discovered at physical examination This is probably an example of metaplasia in metastasis from carcinoma of the breast, although one cannot be positive of metaplasia unless there has been complete examination of the body to make certain that no obscure primary malignant tumor is present

Polyps in the cervical canal of the uterus are rarely malignant and are usually covered with columnar epithelium I have examined cervical polyps which were covered with columnar epithelium in some areas and with squamous epithelium in others with the formation of epithelial pearls (Figs 4 and 5) When tissue of this character appears at operation, two questions must be decided Is the growth a benign cervical polyp with metaplasia of columnar epithelium to squamous epithelium or is it a squamous cell epithelioma developing in a cervical polyp? If the first diagnosis is made, the condition is benign and of little consequence, and removal of the polyp would be sufficient If the second diagnosis is made, the operative procedure should be radical and the prognosis guarded Careful microscopic examination of the tissue in general and of the particular cells reveals no evidence of malignant disease in these polyps and indicates that they are examples of metaplasia of columnar epithelium to squamous epithelium and that the lesion is benign

These cases illustrate some important practical considerations of metaplasia in tissues that sometimes must be considered in the diagnosis of tumors and in the subsequent treatment to be employed

SUMMARY

The change from one type of malignant tissue to another of a related kind gives rise to the question Is this metaplasia or is there more than one primary malignant tumor present? In benign tumors, such as cervical polyps the covering epithelium by means of metaplastic changes may be altered to a different type of related tissue which may simulate a malignant growth although the lesion is actually benign

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FURTHER STUDIES ON THE ORGANISM WHICH PRODUCES SPECIFIC LESIONS IN THE STOMACH AND DUODENUM AND AN INTRA-DERMAL TEST, RELATIVE TO CHRONIC AND LATENT INFECTIONS*

BY ALBERT HOFFMANN,† ANAHEIM, CALIFORNIA

IN A PREVIOUS paper¹ I described an organism (hereinafter called *Bacillus Hoffmanni*) which produced with great regularity, either duodenitis, or ulcer, or both, in the guinea pig, and if injected intramuscularly or if the animal was left to develop a chronic state of the disease, myositis. The object of the present paper is to report what further studies of this organism have revealed.

An organism which can be isolated from man, only with great difficulty, but which, if once isolated in a pure state, grows on the ordinary culture medium very readily, remaining alive for a period of two years without being subcultured, and yet, retaining its specificity, is surely worthy of serious investigation.

A culture grown for nine months in Hiss' serum-water had, aside from a heavy precipitate, a translucent supernatant fluid which upon subculturing into broth media and left at room temperature for a period of twelve months, developed small vesicles the size of lentils which suggested a fungoid growth. Upon microscopic examination only clumps of bacteria, some of them resembling fatty acid crystals, could be seen. Stained smears revealed the same gram-negative organism with which the medium was inoculated.

Of the above culture 2 cc were inoculated intracardiac into a large guinea pig and another received 3 cc intraabdominally. Both animals developed slight symptoms of toxemia after seven hours and both were chloroformed. The one injected into the heart had a deep crater ulcer close to the pylorus and numerous smaller erosions at the cardiac end of the stomach from which blood was oozing. The second animal's stomach was inflamed along the greater curvature and slight duodenitis was present.

This indicates that the twenty-one-months-old culture has lost nothing of its virulence and its elective affinity for the gastric mucosa. The culture was subcultured without passing through an animal. The broth medium within twenty-four hours was cloudy and had a heavy pellicle which at first suggested a contamination. Never before had such a heavy pellicle formed in such a brief space of time. Upon examination nothing could be found except the original organism. The heavy pellicle was shaken down and another one was formed during the next twenty-four hours.

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After growing the organism for five days in broth medium 4 cc were injected intraabdominally at 9 30 A M and another guinea pig received the same quantity of a culture grown for twenty four hours only. Toxemia developed within one hour. The first animal was visibly worse than the second, the latter, however, became very ill later in the afternoon. The five day old culture animal died at 4 P M. The other animal although still alive at 5 30 P M was very sick and was chloroformed.

Necropsy—There were no lesions in the intestines of the first guinea pig. The stomach was hemorrhagic throughout, having deep crater like erosions $\frac{1}{4}$ by $\frac{1}{4}$ of an inch containing blood clots which had partly turned brown in color. In this instance one could sharply differentiate the duodenum from the jejunum the former being severely inflamed while the latter showed not a sign of inflammatory reaction. The pancreas, too, was hemorrhagic. No free HCl was present in the stomach contents, however a strong alkaline odor was noticeable. The second animal which had been injected with the twenty four hour culture had similar lesions but in a milder form. The stomach was not filled with blood clots but had distinct signs of inflammation affecting the serous coat and mucosa the latter having numerous small hemorrhagic areas scattered along the greater curvature.

This is another demonstration of the virulence of this organism brought about simply by subculturing.

The predisposition of the gastric mucosa to peptic ulcer when a large number of the organisms is injected might possibly be explained by the sudden resultant alkalosis. The high degree of alkalinity of the culture medium is doubtless responsible for such a condition. The instantaneous neutralization of the stomach contents and the destructive action of the alkali on the mucosa are seemingly enough to break down the protective barrier allowing the bacteria injected with the already present microorganisms and enzymes to do their destructive work. If the animal survives chronic crater ulcers develop, some of which will heal. This was observed in animals which received small doses. One of them after being injected was in perfect health after six weeks. Necropsy revealed one small active crater ulcer and many healed ulcers. These were seen as white fibroid areas on the outside of the peritoneal coat. At times there has been observed in the guinea pig (after giving an adequate intracardiac dose of the organism) the whole stomach filled with mucus and blood having a nauseous and alkaline odor. All there remained, after inverting the stomach was the muscular and peritoneal coat. From this material, *Bacillus Hoffmanni* was isolated in pure culture by simply seeding some of the mucus upon Russell's double sugar agar.

The individuals suffering from infected foci which harbor this organism in conjunction with others capable of absorbing its toxins and thus acting as intermediary hosts may develop a complex variety of symptoms.

A guinea pig injected with the specific organism showed symptoms of toxemia within half an hour after receiving 4 cc intraabdominally. Seven hours later the animal was chloroformed. From the crater ulcer which was situated close to the pylorus, material was taken and grown in glucose brain

medium² It was also directly seeded upon eosin-methylene-blue agar plates The cultures were examined twenty-four hours later A pure growth of gram-positive streptococci were found in the glucose brain medium, while the agar plates revealed small colonies which upon examination proved to be the same gram-negative organism with which the animal was inoculated (in other words *Bacillus Hoffmanni*), but no streptococcus could be found, nor any other gram-positive organism demonstrated Upon careful examination of the glucose brain medium there were noted some gram-negative cocci and other pleomorphic forms which were, however, attached to the gram-positive streptococci and could hardly be differentiated from them On the second and fifth day the morphologic characteristics of the streptococci did not change

Four c.c. of the above forty-eight-hour-old streptococcus culture were inoculated into a guinea pig weighing 250 grams The animal was chloroformed after three days No symptoms had developed

Necropsy—The duodenum was inflamed and a small ulcer at the cap could be seen The stomach had two minute ulcers at the greater curvature, a large area of the serous coat was inflamed and the stomach on opening, contained gas, and a slight amount of mucus which was adherent to the bulk of the food contents

A second animal was injected with a five day-old culture, an intracardiac injection of 2 c.c. being given The animal was apparently well after twenty-four hours, when it was chloroformed Necropsy revealed similar results as in the guinea pig referred to above as having been chloroformed seven hours after injection Lesions were expected (since I believe that *Bacillus Hoffmanni* was present in the streptococcus culture), but they were mild It is interesting to note that *Bacillus Hoffmanni*, which grows in glucose brain medium luxuriantly and produces lesions in the rabbit (as described in the first paper), was found wanting in ability to reproduce itself What physio-chemic relation exists between this streptococcus and bacillus is not clear to me That the streptococcus acts as a possible repressor, perhaps restricting the virulence of the organism in a being suffering from such an infective process is not unlikely, although it remains still a problem which deserves serious consideration and investigation It has been observed repeatedly that the streptococcus was present in apparently pure culture if grown in glucose brain medium, whether the material was taken from a focus of infection or from a peptic ulcer, but only with difficulty was *Bacillus Hoffmanni* isolated from such material Even when making intramuscular injections with the pure organism producing the typical lesions, I was unable at times to isolate it from the muscle, but upon careful inspection of smears made from the bone marrow I was able to demonstrate the organism microscopically and actually grow it on the ordinary media, reproducing the specific lesions again in subsequent inoculated animals

Up to the present time I have been able, although only with great diffi-

culty, to isolate several strains directly either from foci of infection, such as the nose and the tonsils, or from the stomach contents of individuals suffering from this type of infection

SKIN TEST

An organism possessing such striking pathogenicity for the guinea pig may play an important role in chronic and latent infections

Even before publication of the first paper I was curious to know whether the organism was seriously connected with infective processes in man or was merely of secondary importance. After much deliberation it was decided to try agglutination and precipitation tests. These were abandoned because the results were not striking enough to warrant serious investigation. At last resort was had to the skin test from which no positive results whatever were expected. Should, however, the human skin be able to record a positive reaction in those individuals afflicted with such an infection and a negative reaction in those affected with different ailments or in good health then and only then might one be a little more certain of the organism's pathogenicity in relation to man.

The first test was made on a person who had had nasal catarrh for the last fifteen years and had had four operations and local treatment with but slight relief. For the test there was used a 1 per cent suspension of a seventy-two hour culture in isotonic saline solution, to which was added 0.5 per cent phenol. Generally one can give up to 0.1 cc of a 1 per cent bacterial suspension intradermally without serious effects, but this does not hold with this organism. The patient was given 0.05 cc intradermally. After one half hour a white wheal developed at the point of inoculation but nothing else to justify any kind of conclusion to be drawn. The following day the patient was very ill and had to remain in bed. His arm was swollen and very painful, the erythema extended over the whole arm and down the right side of the chest. The second day was not quite as bad, while on the third he was able to come to the office. His arm was still swollen, the erythema gradually subsiding. The group of doctors who saw this reaction were greatly impressed.

A year later, in 1926 Dr H. R. M. made it possible for the skin test to be given a comprehensive trial. The following is the history of a patient on whom was tried the second skin test.

The patient, Mr. C. aged thirty-one, an oilfield worker, was seen first on June 1, 1926. He complained of indigestion of several months' duration.

Except for a left inguinal hernia repaired in 1917 and an attack of influenza in 1922 the patient had always been in good health. During the past year he had been smoking and chewing a great deal of tobacco. Eight months before coming for examination he had had an attack of indigestion which lasted about four weeks. The present attack had been practically continuous for four and one half months. About one hour after a meal he would have distress and fullness in the epigastrium and a dull burning pain which would last three or four hours or until the next meal was taken when it would be completely relieved only to return in about one hour's time. He also complained of sour eructations of gas and watery fluid accompanying the burning pain. There was no vomiting. About 10 p.m. or later in the evening he would drink a glass of milk as this would relieve the distress and burning for about an hour. Taking bicarbonate of soda also relieved but for a shorter time.

Physical examination was negative except for tenderness in the abdomen over region of the pylorus. Urinalysis was negative. Blood count was normal. Fractional gastric analysis. The fasting stomach contained about 100 cc of fluid, showing free HCl 62° and total acidity 95°. The digestion curve gave a fairly normal level of acidity, but the secretion was prolonged. Fluoroscopic examination of the stomach and duodenum showed hyperperistalsis with a poor filling duodenal cap which showed a notch on its inferior border and a moth eaten appearance on its right side. At the six hour examination the stomach was empty. Diagnosis. Duodenal ulcer.

The skin test was positive after five hours, being characterized by a raised wheal the size of a 25 cent piece and surrounded by slight erythema. This reaction lasted for twenty-four hours without any systemic reaction or discomfort. Dr. H. R. M. and Dr. J. R. were willing to volunteer at once and through their willingness I was able to solicit volunteers for my test. Drs. H. R. M. and J. R. were negative. Shortly after this the discovery was made that a positive reaction could be elicited in patients suffering from asthma, arthritis, hay fever, laryngitis, chronic rhinitis, paranasal sinusitis, colitis, etc. Some reactions were severe, others mild. Those with active focal infections had severer reactions, while others varied accordingly. It seems that each particular illness gives a characteristic reaction. For instance, in asthma those who were positive showed signs of a mild aggravation of symptoms, while others had none. A similar condition was observed in arthritis, etc. It all depended upon the susceptibility of the individual.

From those tested, 19 suffered from arthritis, 8 from peptic ulcer (diagnosed as such either clinically or at operation), 4 from asthma, 3 from hay fever, 7 from chronic rhinitis, 3 from eczema, 6 from colitis, and one patient with exophthalmic goiter having severe tonsillitis. All the above were positive, while 11 out of a total of 62 persons tested, were negative.

It may seem strange that so many positive reactions occur, and one might infer that the organism would elicit a positive reaction in individuals not suffering from this infection at all, but being simply sensitive to the protein and possibly reactive to most any foreign substance thus introduced. However, such reactions can hardly enter, because with rare exceptions the readings are made the next day, that is, twenty-four hours later, some of the severest reactions last as long as seventy-two hours. Others do not make their appearance until the next morning, and those persist for a longer time. Any reaction disappearing before three hours have elapsed is considered negative.

The following history of a patient will illustrate that the organism has a specificity and does not produce a reaction even with triple the quantity of the skin-test injection in individuals suffering from diseases producing a similar clinical picture but due to a different infection. Skin tests were made repeatedly on this patient without success. After study of the bacterial flora of his feces there was isolated a bacterial strain towards which he reacted positively. He improved a great deal after treatment with the autogenous vaccine.

Case No. 2196. March 27, 1926, W. M. P., aged forty six.

Complaints—Abdominal distress and gas belching. Headache.

History of present illness—As a young man, the patient had no digestive trouble, except a tendency to constipation, lassitude, etc. Since having influenza seven years ago, he

had had a good deal of stomach and bowel trouble, characterized by spells of abdominal distress, sometimes amounting to a feeling of soreness over the whole abdomen with gas belching, loss of appetite, lassitude and frontal headache. Attacks lasted usually a day or two and were separated by brief intervals of almost complete relief. They were provoked by slight dietary indiscretion, constipation, worry, or overwork. No constant relation to meals but apples, salads, etc., were aggravating. Three years ago the patient had a spell lasting almost a week with slight jaundice and pain and tenderness over the gall bladder. Operation was performed with removal of gall bladder (strawberry type) and a chronically inflamed appendix. Exploration of the stomach and duodenum was negative. Very little change after operation.

Diagnosis—Chronic colitis

I have observed that in cases where there has been no focus of infection, the reaction was invariably negative. In all cases, however, where the focus existed or had been removed in previous years a positive reaction was obtained.

Many additional cases could be cited but it is deemed advisable to publish them with the necessary clinical data in another paper. In most of the positive cases stock vaccine made from the organism has been administered with results which warrant detailed description.

CONCLUSIONS

1 Further studies revealed that the organism retained its specificity and selective affinity as described previously.

2 Various strains have been isolated either from foci of infection or from the stomach contents from patients suffering from such infections.

3 With 0.01 cc. of a 1 per cent suspension of the organism given intradermally, positive reactions were obtained in 19 cases of arthritis, 8 cases of peptic ulcer, 4 cases of asthma, 3 cases of hay fever, 7 cases of chronic rhinitis (some of which had sinusitis, intrum infections and arthritis), 3 cases of eczema, 6 cases of colitis and 1 case with exophthalmic goiter having severe tonsillitis, 11 cases out of the 62 tested were negative.

4 Although the organism when inoculated into the guinea pig has a selective affinity for the gastric mucosa, etc., it seemingly plays a primary role in those infective processes in man which are chronic and latent. Apparently the organism is living in antagonistic and also possibly constructive symbiosis, thus producing the various symptoms enumerated above.

NOTE—I am indebted to Doctor H. Ross Magee, Anaheim, California, for his interest and helpful criticism.

A STUDY OF THE LARGE DIFFUSE-MARGIN PLAQUE OF SEWAGE FILTRATE*

BY OCCO GOODWIN AND JANET CALDWELL, DALLAS, TEXAS

DURING the isolation of bacteriophage strains from sewage filtrate (S F) an unusually large and peculiar area of lysis was sometimes encountered which differed enough from the classic plaque to warrant a special study.

These lytic areas had been observed at different times with *B. typhosus*, *B. dysenteriae*, and *B. coli*. A stock culture of *B. typhosus* (No. 8) was selected as the organism with which to produce a purified filtrate for study. The filtrate was obtained and purified from these lytic areas by the usual method of plaque picking. One of the clear centers from *B. typhosus* plates, where characteristic areas were well separated, was rubbed with a sterile platinum wire and shaken out into a young broth culture of *B. typhosus*. This was filtered after six hours to remove resistant organisms carried in from the plaque margins, and a second supply of young susceptible organisms was added to a concentration of 3 or 4 billion per c.c. After filtering a second time, a second plating and picking was made, and the fifth serial passage following the second picking was produced in large amount and stored in 10 c.c. quantities for all succeeding experiments. All experiments were performed with this same filtrate 5 (F5) on 17 per cent agar unless otherwise stated.

The characteristic appearance of these areas and their contrast with typical plaques can be noted in the illustration. On ordinary beef extract agar (17 per cent) they are round, 9 to 12 mm. in diameter with perfectly clear sterile centers 5 or 6 mm. in diameter. The outer portion consists of modified culture apparently in the form of minute individual secondary colonies, slightly more opaque and denser than the normal growth but uniform in size, thus giving by reflected light a satiny zone. The outer limit of the area is vague so that the term "diffuse margin plaque strain" is used by the authors in referring to filtrates obtained from these areas.

The questions which the following experiments attempt to answer are whether this lytic area has the essential characteristics of the bacteriophage as described by d'Herelle, or whether it is some other form of lysis, or thirdly, if its characteristics proved to be constant, whether it could be considered a separate bacteriophage thus casting doubt on d'Herelle's contention that there is but one bacteriophage with plurality of races only.

TECHNIC

Agar slants and Petri dishes are unsatisfactory for studies of this lytic principle, because the areas are too large to occur well separated in large

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numbers as is necessary in studies of size and for counting. Therefore, agar was poured in porcelain pans 9 by 5 inches to a depth of 7 mm. The pans were covered with brown wrapping paper and sterilized by dry heat. The standard concentration of organisms in the diluted filtrates was 200,000,000 per c.c., and the mixtures of bacteriophage and suspension were distributed over the surface of the pan again by adding measured amounts with a sterile pipette in the form of a cross and then spreading the fluid uniformly with L shaped glass rods. No especial trouble with contamination was encountered.

Preliminary experiments indicated that isolated plaques could be obtained when pans were spread from serial dilutions of the filtrates using 10^{-4} to 10^{-6} .

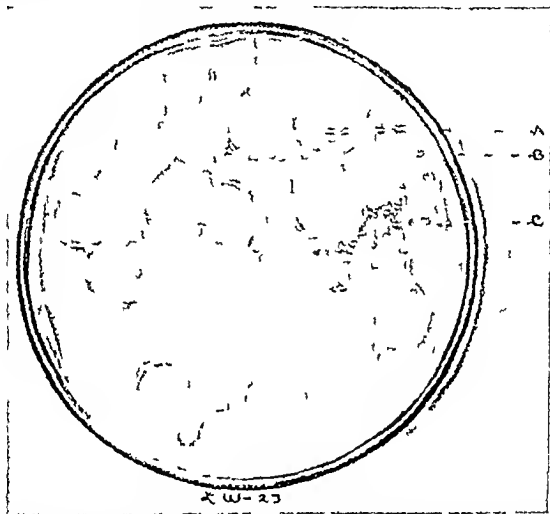


Fig. 1—A Petri dish culture of *B. dysenteriae* No. 14 showing many large diffuse plaques and a few 1 mm. sharp margin plaques. I central sterile area. B outer satiny zone of changed culture. C 1 mm. plaque.

These dilutions were inoculated with organisms to the standard concentration and 0.5 c.c. was the amount usually spread. When filtrates were produced against other organisms which are susceptible to this lytic substance, they were always allowed to reach a maximum concentration of units by giving them a supply of young susceptible organisms at two different times separated by filtration. This is, of course, a necessary part of the technique with all serial dilution work to insure constant results.

SERIAL LYSIS

The original F5 was passed with the stock *B. typhosus* (No. 8) through 25 passages. Lysis was observed in each passage, and pans were spread from the filtrate after each fifth serial passage. The plaques were always

typical and they were not changed, apparently, in concentration, as the same dilutions always gave isolated plaques. Thus, it is apparent that the lytic substance of the large diffuse plaque fulfills the two fundamental requirements of the bacteriophage, i.e., lysis in series and isolated areas of lysis on solid media.

SIZE AND APPEARANCE OF PLAQUES

The appearance of the plaques was practically the same on all concentrations of agar with all organisms against which the filtrate showed lytic action, but the size varied both with the organism used and with the concentration of agar. On standard agar (17 per cent) the smallest plaques were 9 mm in diameter with *B. paratyphosus* B and *B. coli* 98U and the largest was 12 mm in diameter with several organisms. (See Table I.) With the stock *B. typhosus* (No. 8) the plaques were almost all the same size but with certain of the other organisms, where the filtrate was not built up against each but only studied in the first or second contact, the plaques varied down to half the usual size. Then characteristic appearance was, however, always retained and the vague margin and satiny zone with the clear center were conspicuous.

The plaques of F5 were observed on agar of concentration from 0.8 per cent to 3.5 per cent, and they were found to vary consistently being larger the lighter the agar. This is the expected result if these areas behave as do ordinary plaques, for Brousenbrenner¹ has shown that the latter increase in size with decrease in concentration of agar.

TABLE I

VARIATION IN SIZE OF PLAQUE WITH CONCENTRATION OF AGAR AND TIME OF INCUBATION

ORGANISMS	OUTER DIAMETER (MM.)	INNER DIAMETER (MM.)	PER CENT OF AGAR	INCREASE IN DIAMETER		
				48 HR.	72 HR.	96 HR.
<i>B. typhosus</i> 8 Stock	15	9	0.8			
	16	10	1.0	22 mm	28 mm	32 mm
	16	6	1.2			
	12	6	1.7	15 mm	24 mm	
	10	5	2.0			
	8	1	2.5	15 mm		
	7	1	3.5	11 mm	18 mm	
<i>B. typhosus</i> 10 Rawlins	10	3	1.7	15 mm		
<i>B. paratyphosus</i> B	9	1	1.7	10 mm	11 mm	12 mm
<i>B. dysenteriae</i> Shiga	12	6	1.7	17 mm	18 mm	
<i>B. typhosus</i> 17	12	6	1.7			
<i>B. coli</i> 21U	11	6	1.7	16 mm	18 mm	
<i>B. coli</i> 98U	9	4	1.7	12 mm	15 mm	

At no time were ordinary sharp margin plaques seen with these purified filtrates, although dozens of pans and hundreds of plaques were observed during these studies. The fact that the same type of plaque always occurred and alone, indicates that its characteristics are inherent in the lytic substance and are not due to a certain type of resistance in the organisms resulting in unusual secondary colonies. This conclusion is strengthened by the fact that typical sharp margin plaques have been observed with all these organisms when put in contact with sewage filtrate.

Perhaps the most striking observation made during these studies was the fact that the plaques increased in size after twenty four hours. The increase was so marked on 10 per cent and 12 per cent agar that it was noted without measurement and later confirmed by daily measurements. The increase in size was in the outer diameter only as the central, perfectly clear, sterile area remained the same. The outer rim appeared like a slightly depressed line and would often be much more definite twenty four hours after it could be noted and measured. The culture within this rim became similar to the satiny zone but was never as dry or as conspicuous as the original zone immediately surrounding the clear center. The spread still occurred when the concentration of the suspension was increased to 750,000 000 per c c but with 1,000 000 000 or more it was either absent or only slight.

Table I records the increases in diameter from twenty four to ninety six hours. As will be seen in the table the increases occurred with various organisms and on various concentrations of agar, but the increase was greater when the original plaque was large. The figures are the result of several observations with each organism made during other experiments, whenever plaques were well separated and the pans remained free from contaminations. The increases were practically constant. Especially striking were the measurements of *B. paratyphosus B* and *B. coli* 98U whose plaques were always small. In fact it was the small plaque of *B. paratyphosus B* found when using a sewage filtrate (No 5) obtained months after the sewage sample which had been used in obtaining the original large diffuse plaque filtrate that suggested to the authors that this lytic area might be a single entity with a constant type of action on organisms susceptible to it.

As is generally believed in bacteriophage plaques do not increase in size nor is there any change in the culture surrounding plaques after the growth is fully developed, say at twenty four hours and this characteristic spread of the diffuse plaque forms its chief claim to be considered as a separate bacteriophage species.

To determine whether the increase in size was because the lytic substance spread through the culture or whether it was already present at some distance in the normal appearing culture and produced late changes in it filtrates were made from streaks through the culture from 2 to 6 mm from the vague outer border. In every instance lytic action producing similar plaques could be demonstrated in the surrounding culture. Because of drying or late contaminations, these same plaques seldom spread to the point picked but other plaques frequently increased in diameter to this extent. D'Herelle has shown that the bacteriophage penetrates the culture surrounding a plaque over a period of days or even months but it only succeeds in spreading a few mm and no macroscopic changes in the adjacent culture are described by him. With the large diffuse plaque the bacteriophage has been recovered in twenty four hours 15 mm from the outer margin of the inner clear center and this is 6 mm beyond the outer margin of macroscopic change in the culture.

RANGE OF ACTIVITY

The stock F5 was tested with 27 cultures of gram negative bacilli and was found to have lytic action on only 7 (See Table II) These cultures include a few stock cultures of *B. dysenteriae* and *B. typhosus* but most were isolated in this laboratory from cystoscopic specimens of urine or from blood cultures in cases of typhoid fever. Bacteriophage of marked activity has been found for all these organisms in SF, so that the limited range of action of F5 was not due to resistance to bacteriophage action on the part of the organisms.

The organisms which were lysed (*B. coli* 21U and 98U, *B. typhosus* 8, 10 and 17, *B. paratyphosus B* and *B. dysenteriae* 14) were run again with F5 and pans spread until isolated plaques were obtained to see if only the typical appearances occurred or if difference in the organism produced a change in the plaque. Only the diffuse margin plaque was ever found, and it was so constant in its appearance that we felt that we must be dealing with a lytic substance of constant and individual characteristics. To test this idea, some of the susceptible organisms were used to isolate from filtrates of various sewage samples, this same type of lytic area. Samples of affluent city sewage had been collected every two or three months during the past year and portions of SF 2, 3 and 5 were still kept in storage. *B. coli* 21U and 98U and *B. paratyphosus B* were used and purified filtrates produced by the usual process of plaque picking. Most filtrates were purified by 3 or 4 pickings because sharp-margin plaques were also present on the original spreadings but *B. paratyphosus B* SF 5 showed only diffuse-margin plaques from the start and was picked only twice.

Six new purified filtrates were thus produced and these were run against the 27 organisms, and the results are recorded in Table II. In addition to these and the original *B. typhosus* F5 there was in the senior author's notes a record of a diffuse-margin plaque filtrate isolated six months ago with *B. dysenteriae* 14. This record though not complete agreed with the others and is included. For contrast the findings of a filtrate purified from a 3 mm sharp margin plaque with stock *B. typhosus* (No. 8) are included at the end of the table.

The technique of this experiment is as follows. A broth culture was made from a fresh agar slant of each organism and incubated eighteen hours. Two filtrates were tested at a time using 5 to 30 drops of each in two broth tubes, respectively, with a control tube. Thus the racks were set up in sets of 5 broth tubes. The filtrates were pipetted in first and then 3 drops of the broth culture added. Two sterile pipettes were necessary for each organism—one for the first filtrate tubes, the other serving to inoculate the control tube and the second filtrate tubes. Observations were made after three, seven, and twenty-four hours incubation. Agar slants were spread from all 30 drop tubes between the sixth and seventh hours.

There was not one discrepancy in the entire experiment. Every filtrate lysed the same few organisms and was completely negative with the rest. The agreement was perfect even in degree. For instance *B. typhosus* No. 8 was lysed by every filtrate to a maximum degree at three and seven hours.

and showed moderate lysis at twenty-four hours. Twenty one U, however, though perfectly clear through seven hours, showed no lysis at twenty-four hours. None of the other organisms showed more than weak lysis after the seven-hour reading. Apparently early secondary cultures are the rule with this bacteriophage, and despite the large size of the plaques and the constancy with which sterile agar slants are obtained it is obviously inferior to sharp margin plaque strains where permanent marked lysis can usually be obtained with this same group of organisms. Even after the 25 passages with B typhosis no improvement in the degree of clearing at twenty-four hours occurred so that its virulence is not built up rapidly as are most bacteriophage strains obtained from S.F. This plaque is an exception to the rule that large plaques mean a virulent bacteriophage, for although the plaque is large with a variety of organisms, with none of them is a really satisfactory permanent lysis obtained. The same type of agar slants occurred, usually they were practically sterile with only scattered colonies varying in size. Ragged edges do not occur on slants with this bacteriophage as the plaques are so large that when fused the small area of a slant is sterilized, and only the few resistant organisms form scattered colonies over its surface.

We have at present no explanation to offer for the peculiarities of the lytic substance described, but investigation of it has by no means been exhausted. It was at first supposed that it might be a larger particle or be carried by larger particles than ordinary bacteriophage units, but it passed readily through collodion sacs of the following composition:

Parlodion—2 per cent in equal parts of ether and 95 per cent alcohol

Parlodion—3.5 per cent in 2 parts of ether and 1 part 95 per cent alcohol

Still less permeable membranes should be tried to see whether or not a membrane could be found which would let ordinary plaque producing strains through but retain this substance. This possibility was suggested by the work of Bronfenbrenner who has succeeded in fractionating different sized plaque races of bacteriophage by ultrafiltration.

DISCUSSION

As has already been stated the lytic substance just described has two of the fundamental characteristics of the lytic principle described by d'Herelle. It produces lysis in series and in isolated areas, plaques. Thus the substance must be admitted as belonging to the class of phenomena called bacteriophage.

It differs in that it is much larger than any plaque noted by d'Herelle or hitherto described and it has a characteristic outer zone of changed culture which spreads to enlarge the outer diameter over a period of days. The other distinctive feature of the substance brought out in this paper is that it is apparently an entity whose characteristic size, appearance, and range of activity are always the same, no matter from what sewage sample it is fished nor with what susceptible organism it is isolated.

Whether it is only one of the races of the bacteriophage or a separate bacteriophage species, perhaps cannot be answered by these studies, but the variation from type of this substance is different from the variations claimed

by others as bases for forming separate bacteriophage species. In his latest book d'Herelle⁴ summarizes these claims. These, briefly, include difference of various bacteriophage strains in antigenic properties, difference in resistance to various harmful reagents, the well known fact that required resistance to one bacteriophage may be accompanied by sensitiveness to a strain previously ineffective on the culture, and, finally, difference in form of lytic colony. The latter contention up to the present has meant only difference in size of plaques in which few would dispute d'Herelle that it could scarcely be the basis for separate bacteriophage species depending as it does on so many variables such as virulence, nature of growth of organism, concentration of agar.

D'Herelle does not accept any of these claims as sufficient for establishing the plurality of the bacteriophage and answers well the arguments advanced by their proponents. None of these claims involve variations in fundamental characteristics of the bacteriophage which could not be accounted for by adaptation unless possibly, it is the variation in antigenic properties. As the suitable separation of the bacteriophage from bacterial proteins has only been accomplished recently, it is probable that these variations were due to traces of bacterial proteins of distinct antigenic quality in the filtrates. At least such work will have to be confirmed with bacteriophage solutions purified according to recent careful methods.⁵

The substance here described does differ, however, in one of the accepted essentials of bacteriophage phenomena—the permanency of the plaque in size. D'Herelle and others lay much stress on this permanency and also consider the sharp outer margin as a constant feature. When we consider the striking peculiarities in appearance of the diffuse margin plaque and couple this with the proved singleness of the substance we believe that it has a greater claim as a separate bacteriophage species than any previously advanced claim.

SUMMARY

A plaque has been described which averages 12 mm in diameter and which has an outer zone of changed culture that increases gradually in size to enlarge the diameter of the plaque as much as 100 per cent.

The plaque has been isolated from several samples of sewage collected during a nine month period and all these plaques have identical characteristics and their filtrates have the same range of activity in a group of twenty seven bacilli.

The peculiarities and singleness of the substance are believed to be sufficient basis for considering it as a separate bacteriophage species.

NOTE. Hadley has described similar lytic areas in a culture of paratyphoid bacilli (Proc Soc Exp Biol and Med, February 1928 *xxv* 309) and finds that ordinary plaques do occur in pure line cultures of the large plaques. He considers the two types to be different components of the bacteriophage.

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- ²D'Herelle, F. The Bacteriophage and Its Behavior. Baltimore, 1926. Williams and Wilkins, p. 94.
- ³Bronfenbrenner, J. Jour Exper Med, 1927, *xlii* 873.
- ⁴Ibid, p. 366.
- ⁵Weiss, Emil. Jour Immunol, 1927, *xiii* 311.

TABLE I

DATE	P R (SECONDS)	HEART RATE	REMARKS
January, 1926	0 34		Taken in another laboratory
April 14, 1926	0 28	80 89	
April 14, 1926	0 18	119 121	30 minutes after 1/30 gr atropine
April 20, 1926	0 26	62	
April 20, 1926	0 26 0 32	58 62	Pressure on left vagus
April 20, 1926	0 26 0 32	58 65	Pressure on right vagus
June 15, 1926	0 24 0 28	58 63	
June 29, 1926	0 24 0 28	58 61	
August 8, 1926	0 21 0 22	71 78	Taken in Cleveland, Ohio, by Dr R W Scott
September 9, 1926	0 22 0 24	72 74	
June 15, 1927	0 24	70	
September 27, 1927	0 24	73 83	
September 27, 1927	0 20	104 109	After one minute of strenuous exercise
September 27, 1927	0 21 0 24	83 86	One minute later

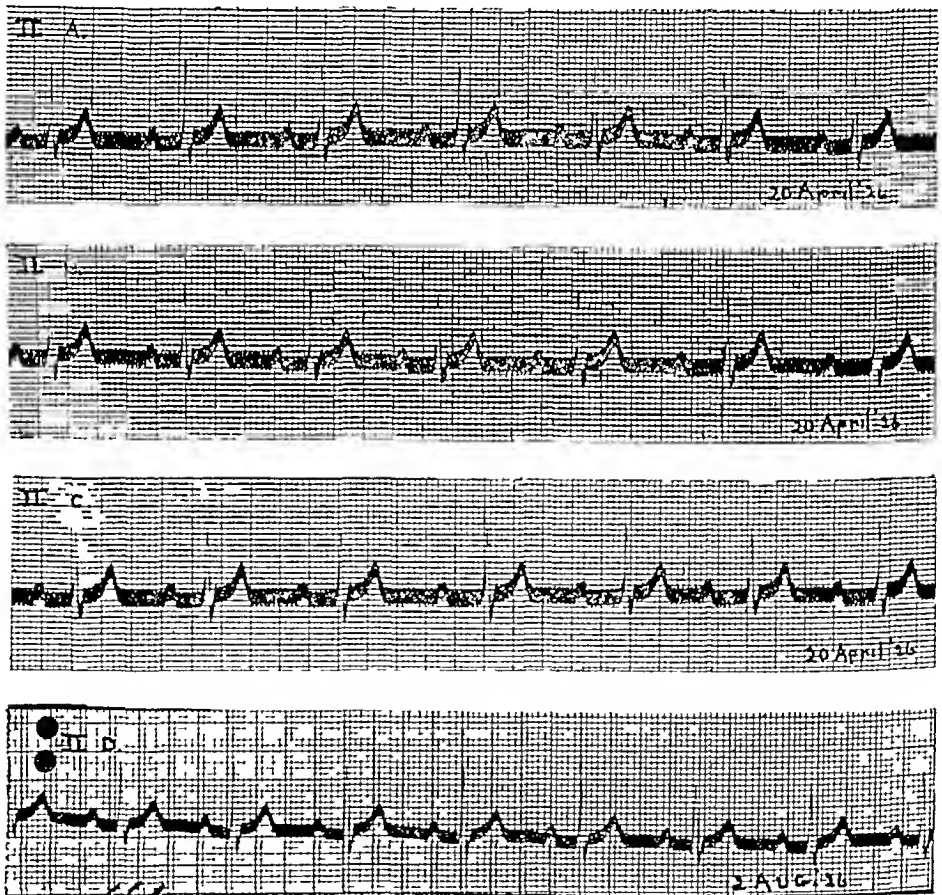


Fig 2—Four records of Lead II dates on prints A Normal conditions P-R=0 26 sec Heart rate=62 B, During pressure on left vagus P-R=0 26-0 32 sec Rate=58 62 C, During pressure on right vagus P-R=0 26-0 32 sec Heart rate=58-65 D Normal conditions P-R=0 21-0 22 sec Heart rate=71-78 (Courtesy of Dr R. W. Scott.)

to the bundle of His, as a result of direct interference with the intrinsic cardiac circulation by the pressure of the effusion on the coronary arteries and veins

Gager refers to a few other reports concerning changes in the blood supply causing partial heart block, and believes such changes may explain some cases of transient or even more definite heart block in which at autopsy the bundle of His may be found to be essentially normal



Fig 3.—Three records of Lead II all taken Sept. 2, 1927. A Normal conditions. P-R = 0.24 sec. Heart rate = 73.38. B Immediately after exercise. P-R = 0.26 sec. Heart rate = 104.109. (Rise in the base line of the atrial shadow is due to shifting of the position of the heart during the postexercise dyspnea.) C and C' Directly continuous record taken one minute after B. The P-R shows a gradual lengthening from 0.21 to 0.24 sec. Heart rate = 8.86.

Maron and Winterberg³ describe a case of rheumatic heart disease with mitral stenosis in which partial heart block with marked variation in the duration of the P-R interval was recorded. They found the following variation:

P-R = 0.46 second	pulse rate 75	Dec 4, 1920
" = 0.40 "	" 62	" 10 "
" = 0.25 "	" 69	" 12 "
" = 0.21 "	" 66	" 20 "
" = 0.44 "	" 71	" 30 "

Immediately after exercise this P-R shortened to 0.18-0.19 second but increased after the next few beats to 0.50 second and then varied between 0.27

TABLE I

DATE	P R (SECONDS)	HEART RATE	REMARKS
January, 1926	0 34		Taken in another laboratory
April 11, 1926	0 28	80 89	
April 14, 1926	0 18	119 121	30 minutes after 1/30 gr atropine
April 20, 1926	0 26	62	
April 20, 1926	0 26 0 32	58 62	Pressure on left vagus
April 20, 1926	0 26 0 32	58 65	Pressure on right vagus
June 15, 1926	0 24 0 28	58 63	
June 29, 1926	0 24 0 28	58 61	
August 8, 1926	0 21 0 22	71 78	Taken in Cleveland, Ohio, by Dr R W Scott
September 9, 1926	0 22 0 24	72 74	
June 15, 1927	0 24	70	
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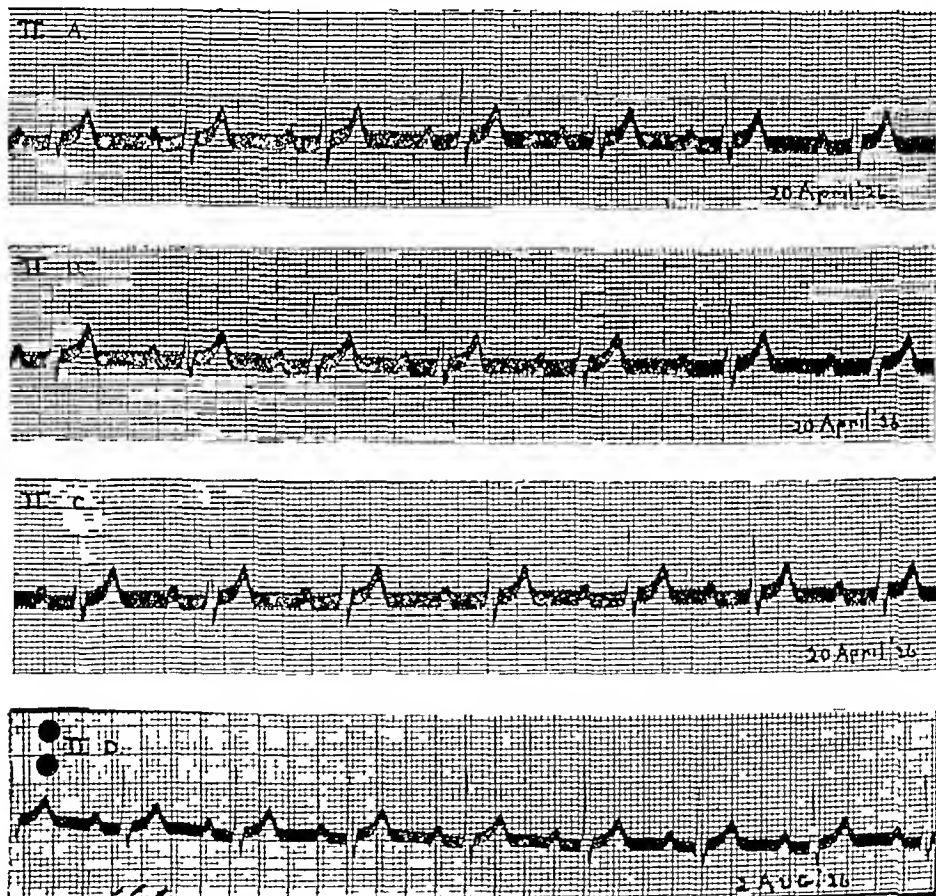


Fig 2—Four records of Lead II dates on prints A, Normal conditions P-R=0 26 sec Heart rate=62 B During pressure on left vagus P-R=0 26-0 32 sec Rate≈58 62 C, During pressure on right vagus P-R=0 26-0 32 sec Heart rate=58-65 D Normal conditions P-R=0 21-0 22 sec Heart rate=71-78 (Courtesy of Dr R W Scott)

to the bundle of His, as a result of direct interference with the intrinsic cardiac circulation by the pressure of the effusion on the coronary arteries and veins

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Fig 3—Three records of Lead II all taken Sept. 27 1927 A Normal conditions P-R = 0.24 sec. Heart rate = 73.38 B Immediately after exercise P-R = 0.0 sec Heart rate = 104.109 (Rise in the base line of the string shadow is due to shifting of the position of the heart during the postexercise dyspnea.) C and C' Directly continuous record taken one minute after B The P-R shows a gradual lengthening from 0.1 to 0.24 sec Heart rate = 83.86

Maron and Winterberg³ describe a case of rheumatic heart disease with mitral stenosis in which partial heart block with marked variation in the duration of the P-R interval was recorded They found the following variation

P-R = 0.46 second	pulse rate 75	Dec 8 1920
" = 0.40	72	10
" = 0.25	69	12
" = 0.21	66	20
" = 0.44	71	30

Immediately after exercise this P-R shortened to 0.16-0.19 second, but increased after the next few beats to 0.50 second, and then varied between 0.27

and 0.28 second. This rapid return to a prolonged P-R is similar to the case I am reporting.

A case somewhat more similar to my case is that of a medical student⁴ devoted to out-of-door sports, especially skiing. He had never noted any signs of heart disturbance during strict physical training and nothing abnormal was found on clinical or x-ray examination, yet electrocardiograms disclosed a P-R interval of almost twice the normal duration. Under the effect of exercise and atropine, the heart-block disappeared entirely.

In the case I am reporting, it is not clear why such variation in the P-R interval occurs. Its reduction to 0.18 second, which is within the limits of the normal duration, does not, as is well established, prove that the delay is necessarily solely dependent upon the influence of the vagus, the latter does not usually produce a prolongation to the degree or as persistent as that found in my patient. The bundle of His may not be normal, and yet under ordinary conditions may still conduct within the normal time limits. In such a case, however, the P-R interval may be lengthened to an abnormal period by the influence of the vagus upon a bundle of His which is already imperfect in function. It is conceivable that in this case the conducting bundle is congenitally imperfect or may have received some injury from an unrecognized infection, the variations in the duration of the P-R interval may then be due to variations in the vagus influence.

The observation of this patient serves to modify somewhat the rather serious interpretation usually applied to the finding of partial heart-block.

SUMMARY

A case of partial heart-block with considerable variation in the length of the P-R interval, and occasional reduction to a normal duration spontaneously, after atropine, and immediately after exercise, is reported. The history and physical examination give no evidence of abnormality, the patient apparently is in good health.

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MORPHINE TOLERANCE*

I THE ACQUIREMENT, EXISTENCE AND LOSS OF TOLERANCE IN DOGS

BY AROREY W. DOWNS AND NATHAN B. EDDY, EDMONTON, ALBERTA, CANADA

IN preparation for experiments reported elsewhere¹ upon the susceptibility of morphine tolerant animals to allied drugs and to cocaine, we habituated six dogs to large doses of morphine. In each case the initial dose was 50 mg. of the sulphate per kilogram of body weight. This was increased as the condition of the animal warranted until a dose of 150 mg. per kilogram was reached. The salt was dissolved in 0.9 per cent sodium chloride solution 50 mg. per 1 c.c., and administered subcutaneously. The injections were made daily except Sunday. The usual increase in the dose was 10 mg. per kilogram and two weeks were required to work up to the maximal dose which was continued for three weeks. One of the dogs early showed marked resistance to the drug and in her case the maximum dose was increased to 200 mg. per kilo. One dog died on the twenty first day of the experiment and another on the twenty fifth day. The dog that died first had received the maximal dose four times and the other nine times. The cause of death could not be determined in either case.

TABLE I
INITIAL DOSE

DOG NO.	BEHAVIOR		PUPIL		PULSE		RESPIRATION		TEMPERATURE		
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	
			MM.	MM.					C.	C.	
15	Active	Asleep	8	5	130	108	15.0	40	38.8	39.5	Defecated
20	Active	Drowsy	6	6	120	70	42	95	39.7	39.2	Vomited
42	Quiet	Asleep	6	5	91	64	20	46	39.6	39.5	
45	Restless	Quiet	7	8	135	96	43	150	39	39.0	Vomited
93	Restless	Asleep	7	6	150	48	18.5	64	40.0	40.0	
99	Active	Drowsy	9	7	120	96	18	160	39.0	39.2	Vomited
Average			7.1	6.1	126	77	—	95	39.4	39.2	

We adopted the degree of narcotic effect as the index of the action of the drug. In addition the width of the pupil, pulse rate, respiratory rate, and temperature were recorded before the administration of the morphine and thirty minutes after. These observations on each dog before and after the injection of the initial dose are set forth in Table I, before and after the first maximal dose in Table II, and before and after the last dose in Table III. Comparison of these tables shows adaptation of the mechanisms controlling vital activities to the action of the drug. The effects of a dose of morphine after the dogs had become accustomed to a fairly large dose were similar in kind to those produced by the initial dose, though usually less in degree.

From the Department of Physiology and Pharmacology, University of Alberta.
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TABLE II
FIRST MAXIMAL DOSE

DOG NO	BEHAVIOR		PUPIL		PULSE		RESPIRATION		TEMPERATURE	
	BEFORE	AFTER	BEFORE MM	AFTER MM	BEFORE	AFTER	BEFORE	AFTER	BEFORE ° C	AFTER ° C
15	Normal	Slightly drowsy	8	6	100	132	12	15	38.7	38.0
20	Quiet	Quiet	8	8	76	96	15	18	38.9	38.2
42	Quiet	Walking about	6	4	72	76	16	20	38.7	38.5
15	Quiet	Quiet	7	7	100	104	27	26	39.4	39.4
93	Normal	Restless	7	7	124	120	18	28	39.5	39.2
99	Slightly depressed	Slightly depressed	9	9	88	116	12	16	38.6	38.8
Average			7.5	6.8	93	107	17	20	38.9	38.6

Defecated
Defecated
ConvulsionTABLE III
LAST DOSE

DOG NO	BEHAVIOR		PUPIL		PULSE		RESPIRATION		TEMPERATURE	
	BEFORE	AFTER	BEFORE MM	AFTER MM	BEFORE	AFTER	BEFORE	AFTER	BEFORE ° C	AFTER ° C
15	Quiet	No depression	7	5	99	76	18	16	39.0	38.6
20	Quiet	Quiet	9	8	81	110	21	30	39.0	38.8
42	Quiet	No depression	4	4	118	86	20	16	39.1	38.6
15	As usual	No depression	8	7	122	110	38	30	39.5	38.9
93	As usual	No depression	7	10	160	138	10	26	39.8	39.8
99	As usual	No depression	9	8	92	136	25	23	38.9	38.7
Average			7.3	7	112	108	22	23	39.2	38.9

The initial dose produced depression of psychical activity. If roused and made to move about, each dog showed marked weakness and incoordination in the hind limbs. This was not noticed in any animal after the third injection. As the treatment continued all the dogs became quieter but not particularly more drowsy after the injection than before. Dogs 15 and 45 showed a decided change in character which was especially marked in Dog 45. Her manner became slinking and furtive in contrast to the free and rather disdainful fashion in which she had trotted about the room at first. Dogs 15, 42 and 45 quite evidently had hallucinations. We were never able to detect any desire for the injection on the part of any of the dogs. Nevertheless the effect of abstinence from the drug for even one day was pronounced. On Monday morning the dogs were always much more active than usual.

Sollmann states that in the dog the effect of morphine on the pupil is variable but generally dilator. After the first dose of morphine we found the pupil to be constricted in four of the six dogs, unchanged in one and slightly dilated in one. The average reduction in the size of the pupil was 14 per cent. The last dose of morphine also constricted the pupil slightly in four of the six dogs, left it unchanged in one and dilated it in one. The average reduction in the diameter now was only 4 per cent.

The first dose of morphine decreased the pulse rate markedly 38.88 per cent. Later the heart rate averaged less before the giving of the morphine than at the beginning of the experiment. At the time of administration of the first maximal dose the decrease was 26 per cent. This might have been due to persistent effect of the morphine or it might have been the result simply of the quieter behavior of the animals at this time. However thirty minutes after the injection of the maximal dose of morphine the pulse rate increased in all but one animal. The average result was an increase of 15 per cent.

Van Edmond³ claims that in the habituation of dogs to very large doses of morphine the vagus center retains its responsiveness to morphine almost unchanged.

Recently McRea and Meek⁴ have reported experiments tending to show that the slowing of the heart by morphine is not more than partly due to direct stimulation of the vagus center but that it is rather principally secondary to the depressant effect of morphine on the brain. If we accept their view it may be that the vagus center does fail to become completely tolerant to morphine and that absence of the slowing effect upon the pulse in the habituated animal is due to absence of the depressant effect on the brain.

Heinckamp⁵ has observed that adrenalin produces a greater degree of slowing of the heart after the previous injection of a small dose of morphine. He attributes this to synergism between morphine and adrenalin on the vagus center. Persistence of this synergistic action in an animal habituated to morphine should indicate lack of tolerance on the part of the vagus center. Accordingly we administered adrenalin to three tolerant dogs and to three control dogs. Each received an intramuscular injection of 0.1 cc per kilogram of body weight of adrenalin chloride solution 1:1000. The pulse rate was noted before the injection and for the ten minutes immediately follow-

TABLE IV
EFFECT OF ADRENALIN ON THE RATE OF THE HEART

DOG NO	NORMAL DOGS				DOG NO	HABITUATED DOGS			
	BEFORE MORPHINE BEFORE ADRENALIN	AFTER ADRENALIN	BEFORE ADRENALIN	AFTER MORPHINE AFTER ADRENALIN		BEFORE MORPHINE BEFORE ADRENALIN	AFTER ADRENALIN	BEFORE ADRENALIN	AFTER MORPHINE AFTER ADRENALIN
1	156	122.7	108	97.3	15	128	117.9	144	128.4
2	120	136.1	120	103.9	20	81	88.0	110	91.0
3	96	93.8	72	48.7	93	160	153.0	138	143.5
Average	124	117.5	100	83.3	Average	123	119.6	130.6	120.9
Change in per cent		-5.24		-16.7	Change in per cent		-2.76		-7.42

ing The habituated dogs then received their usual dose of morphine and a second dose of adrenalin thirty minutes later. The pulse rate was counted as before. The dose of morphine sulphate given the control animals was 10 mg per kilogram. This was followed by the same dose of adrenalin as given previously. The counting of the pulse rate was carried out as described. The average pulse rates per minute are given in Table IV. From these results it would seem that the vagus center is still responsive in the habituated dogs to the stimulating action of adrenalin and of the increased blood pressure due to adrenalin and also that after morphine the vagus center in both normal and habituated dogs is more sensitive than before.

On the following day the dogs were much more depressed than usual and two, Nos 20 and 93 died. The death of these dogs was mentioned early in this report and the statement made that the cause of death could not be determined. Two possibilities present themselves. The adrenalin may have broken their tolerance to morphine so that the dose of 150 mg of morphine sulphate per kilogram of body weight was more than they could stand or the adrenalin may have sensitized the nerve centers so that 150 mg became equivalent to some much larger dose. Dog 15 was aroused with difficulty the next morning and no morphine was given. On the following day 50 mg caused very great depression, but during the next four days it was possible to return gradually to the usual dose of 150 mg per kilogram.

Two weeks after morphine had been discontinued the three survivors among the habituated dogs Nos 15, 42 and 99 and two of the control dogs, Nos 1 and 2, were given adrenalin chloride solution of the same strength and in the same dose as before. The dose was repeated after forty five minutes, but the morphine was omitted. No depression was observed in any animal. Still later, five weeks after the withdrawal of morphine the same dogs were given a single similar dose of adrenalin chloride solution followed immediately by morphine. The dose of morphine sulphate for the control dogs was 10 mg per kilogram of body weight and for the others 50 mg per kilogram. The results were compared with those of a single similar dose of morphine in the control dogs and of the initial dose in the others. We were unable to detect any greater depression than with morphine alone.

There was one effect of the intramuscular injection of adrenalin which should be noted. In every case there was a local reaction—the leg became swollen and painful and in most instances a discharge of necrotic tissue began on the seventh or eighth day. Probably the necrosis was due to constriction of the arterioles at the site of injection. Biberfeld⁶ reports the subcutaneous injection of protein (milk) as effecting a temporary break in the tolerance of dogs to successive doses of morphine. This leads us to wonder whether the great depression produced by morphine and adrenalin in the habituated dogs might have been due to a break in tolerance caused by necrosis and the entrance of protein degradation products into the circulation.

Morphine always reduced the body temperature. The reduction was about the same whether we consider the effect of the first or of the last dose.

Vomiting occurred in three of our dogs after the first dose of morphine only. Later, about the time the maximal dose was reached or shortly

all the dogs became salivated. This condition persisted until after the withdrawal of the morphine. This might indicate tolerance of the vomiting center to the depressant effect of morphine. Apomorphine was employed to test the possible existence of such tolerance.

Three normal dogs were selected and given apomorphine subcutaneously. The apomorphine was dissolved in normal saline to make a 0.1 per cent solution. Two of the dogs received 0.2 mg apomorphine per kilogram of body weight and one received 0.4 mg per kilogram. All vomited. The average time until the first vomiting was six minutes, fifty-seven seconds, and the average number of times a dog vomited was twenty-five. Five days later the same dogs were given morphine sulphate, 10 mg per kilogram, and forty-five minutes afterward 0.2 mg apomorphine per kilogram. None vomited after the apomorphine.

The dogs that had been habituated to morphine were given their morphine and thirty minutes later 0.2 mg of apomorphine per kilogram of body weight. All vomited. The first vomiting occurred at an average interval of eight minutes, thirty-one seconds, after the apomorphine and the average number of times each vomited was five. A comparison of these results in normal dogs without and with morphine and in dogs that had been receiving morphine regularly justifies the conclusion that the dogs of the latter group were tolerant to morphine in the amounts administered.

All of the dogs lost weight rapidly during the first week. This was principally because they were so depressed that they ate little. However, from the second week on their weight remained stationary or showed a slight gain.

TABLE V
CONDITION OF SURVIVING DOGS TWO WEEKS AFTER LAST DOSE OF MORPHINE

DOG NO	BEHAVIOR	PUPIL MM	PULSE	RESPIRATION	TEMPERATURE ° C
15	Active	5	148	24	38.9
42	Active	4	136	28	39.0
99	Active	5	152	30	39.1
Average		4.7	145	27	39.0

When the morphine was withdrawn, no attempt was made to reduce gradually but there was simply a sudden stoppage of the daily dose. Previous to this Dog 45 had been killed by cocaine. The three survivors showed no ill effects of this drastic procedure but instead manifested an increasing liveliness, so that at the end of two weeks they were all restored to their original condition in this respect (Table V). Salivation persisted five days after cessation of the morphine in one and eleven days in each of the others.

SUMMARY

Dogs habituated to the daily subcutaneous injection of morphine sulphate, 150 mg per kilogram, showed a high degree of tolerance to the depressant action of the drug on the cerebrum and on the vomiting center. Tolerance to the action of morphine on the heart rate and on the size of the pupil was also shown. Body temperature was always lowered by morphine.

We are indebted to Mr J P Quigley for assistance in making the observations recorded in this report

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MORPHINE TOLERANCE*

II THE SUSCEPTIBILITY OF MORPHINE TOLERANT DOGS TO CODEINE HEROINE AND SCOPOLAMINE

By AROREI W DOWNS AND NATHAN B EDDY, EDMONTON ALBERTA, CANADA

DOGS which had been rendered tolerant to morphine as described previously¹ were subjected to the action of codeine heroine and scopolamine in the hope that it might be possible to arrive at some conclusion in regard to the existence of cross tolerance

Codeine—Two normal dogs were first given codeine and subsequently three morphine habituated dogs were given codeine. The dose for one of the control animals was 20 mg of codeine phosphate per kilogram of body weight and for the other 40 mg. Two of the tolerant dogs had been receiving 150 mg of morphine sulphate per kilogram of body weight daily. To one of them 40 mg of codeine phosphate per kilogram was given and to the other 80 mg. The third morphine habituated dog was accustomed to 200 mg of morphine sulphate per kilo and she was given 100 mg of codeine phosphate per kilogram. The drug was made up in 5 per cent solution and given subcutaneously.

The effect in the control dog of 20 mg of codeine phosphate per kilogram was drowsiness and very rapid and irregular respiration. The pupil was constricted. Pulse rate and temperature were slightly increased. One hour and forty minutes after the injection the dog was about as usual. Forty milligrams of codeine phosphate per kilogram caused marked disturbance of equilibrium, increased spinal reflexes, and very rapid respiration. The pupil was dilated, and the pulse rate was increased. One hour and forty minutes after the injection the dog was sleeping lightly and five hours after injection was still slightly depressed.

In the dog habituated to morphine 40 mg of codeine phosphate per kilogram of weight caused an increase in spinal reflexes and rapid, irregular respiration. Pupil and pulse rate were unchanged. Temperature was slightly increased. Gait was normal. At the expiration of one hour and forty minutes the dog was practically normal. The larger doses of codeine in the morphine habituated dogs caused unstable equilibrium, an increase in spinal

reflex activity, very rapid respiration, dilatation of the pupil, an increase in pulse rate and a rise of temperature. The dog which received 100 mg of codeine phosphate per kilogram had a convulsion forty minutes after the drug was administered. One hour and forty minutes after injection both were practically as usual.

These results show a decided increase in tolerance to codeine as the result of habituation to morphine. A comparison of the effect of the 40 mg dose in the two cases brings out clearly the difference as regards narcotic action. In the control dog this dose caused very definite disturbance of equilibrium and sleep. In the morphine tolerant dog there was no effect on equilibrium and no drowsiness. The protocols of these two experiments follow.

<i>Normal Dog</i>		<i>Morphine Dog</i>	
Dog 1—male, 22.25 kg		Dog 99—female, 14.3 kg	
May 12, 1926		May 13, 1926	
10 35 A M	Lively	10 02 A M	Very active
	Pupil 9 mm		Pupil 11 mm
	Pulse 100		Pulse 122
	Respiration 23		Respiration 15
	Temperature 39.6°		Temperature 39.1°
10 40 A M	Codeine phosphate (5% solution)	10 06 A M	Codeine phosphate (5% solution)
	40 mg per kilo		40 mg per kilo
10 49 A M	Unsteady on legs	10 39 A M	Marked increase in spinal excitability on stimulation
10 55 A M	Lying down		Pupil 10 mm
11 02 A M	Panting		Pulse 122
11 07 A M	Disinclined to walk. Marked disturbance of equilibrium		Respiration 34, irregular
	Salivated		Temperature 39.5°
11 13 A M	Spinal reflexes very active	10 40 A M	Vomited
	Pupil 11 mm		Gait practically normal
	Pulse 126	11 55 A M	Practically normal
	Respiration 60	3 00 P M	Same
	Temperature 39.5°		
11 23 A M	Respiration 208		
12 20 P M	Gait improved		
	Sleeping lightly		
3 15 P M	Only slightly depressed		

None of the morphine tolerant dogs was caused to sleep or even rendered drowsy by codeine. To produce the other effects in like degree in the morphine tolerant dogs as in the nontolerant, the dose had to be doubled.

Heroin—Two normal dogs were given heroin on two successive days. A 0.1 per cent solution of the hydrochloride in normal saline was prepared and given hypodermically. On the first day each dog received 0.3 mg per kilogram of body weight and on the second day 0.6 mg. The only noticeable effect of the smaller dose was on respiration, which became rapid and irregular. The larger dose caused very rapid and irregular respiration and sleep.

Our three morphine tolerant dogs were given doses of heroin ten times as large as those given to the nontolerant dogs without any effect upon respiration. One dog slept lightly after 3 mg of heroin hydrochloride per kilogram, but did not go to sleep after 6 mg. The others showed no depression with either dose.

All of the dogs both tolerant and nontolerant to morphine, failed to show any effect upon gait or spinal reflexes with the doses used. Pulse rate and temperature were either not affected or were slightly decreased. The pupil was unaffected. A typical protocol from each group is submitted.

<i>Normal Dog</i>		<i>Morphine Dog</i>	
Dog 2—female 17.5 kg		Dog 42—male, 18.25 kg	
May 11, 1926		May 11 1926	
10 08 A.M.	Pupil 12 mm	10 20 A.M.	Pupil 5 mm
	Pulse 132		Pulse 105
	Respiration 70		Respiration 17
	Temperature 40.3		Temperature 39.2
10 10 A.M.	Heroin hydrochlor (0.1% solution)	10 23 A.M.	Heroin hydrochlor (0.1% solution)
	0.6 mg per kilo		6 mg per kilo
10 20 A.M.	Drowsy	10 33 A.M.	Very little, if any depression
10 45 A.M.	Sleeping		Pupil 6 mm
	Gait unaffected		Pulse 80
	Pupil 11 mm		Respiration 16
	Pulse 104		Temperature 38.6
	Respiration 130 and irregular	1 30 P.M.	Running about
	Temperature 39.5		No depression
12 20 P.M.	Almost normal	5 00 P.M.	Same
1 30 P.M.	Depression very slight		
5 00 P.M.	Normal		

Scopolamine—Scopolamine hydrobromide in 1 per cent solution was administered to three normal dogs. The dose was 0.67 mg per kilogram of body weight. The only effect apparent was moderate depression. When the same dogs received the same dose of scopolamine followed thirty minutes later by morphine sulphate, 10 mg per kilogram they went to sleep twenty minutes after the morphine and could be roused only partially five hours later. The next day they still showed some depression. Ten milligrams per kilogram of morphine sulphate alone caused two of these dogs to vomit and all to sleep lightly. They went to sleep in twenty minutes and were drowsy five hours later.

When the morphine tolerant dogs were given the same dose of scopolamine hydrobromide 0.67 mg per kilogram, they first became restless. Then they manifested unsteadiness in gait, walked with legs stiff and failed to avoid objects. One became quite crazy, howling and barking and bumping into tables and the wall. Thirty minutes after the scopolamine each received his usual dose of morphine. In all depression was much more marked than usual though they could be roused. Two vomited. All were as usual the next day.

The two protocols given below are indicative of the results obtained with scopolamine and morphine.

<i>Normal Dog</i>		<i>Morphine Dog</i>	
Dog 1—male 22 kg		Dog 99—female 13.5 kg	
May 8 1926		May 8 1926	
0 42 A.M.	Lively	9 23 A.M.	Quiet Trembling
	Pupil 11 mm		Slightly irritable
	Pulse 128		Pupil 10 mm
	Respiration 27		Pulse 136
	Temperature 39.8		Respiration 12
			Temperature 39.0

9 45 A M	Scopolamine hydrobromide (1% solution) 0.67 mg per kilo	9 32 A M	Scopolamine hydrobromide (1% solution) 0.67 mg per kilo
10 12 A M	Depression slight A little restless Pupil 12 mm Pulse 196 Respiration 130, panting Temperature 39.8°	9 43 A M	Restless
10 15 A M	Morphine sulphate (2% solution) 10 mg per kilo	9 45 A M	Unsteady on feet Stiff legged gait Does not seem to know where she is going
10 22 A M	Unsteady on feet Panting	9 50 A M	Considerable depression
10 23 A M	Lying quietly	10 00 A M	Pupil 12 mm Pulse 172 Respiration 30 Temperature 39.2°
10 27 A M	Hard to rouse	10 03 A M	Morphine sulphate (5% solution) 150 mg per kilo
10 32 A M	Sleeping	10 10 A M	Very marked depression
10 39 A M	Sleeping Pupil 12 mm Pulse 184 Respiration 180 Temperature 38.8°	10 27 A M	Depression much more marked than usual Twitching Pupil 13 mm Pulse 200 Respiration 110 Temperature 38.8°
12 00 M	Rouses slightly on strong stimulation	10 48 A M	Vomited
3 00 P M	Can be partially roused	12 00 M	Rouses slightly on strong stimula- tion
May 9		3 00 P M	Moderate depression Easily roused
11 00 A M	Still some depression	May 9	
		11 00 A M	As usual

Cushny² states that dogs which have been rendered tolerant to morphine are equally refractory to its allies, codeine and heroine. Blair³ expresses the belief that morphine and heroine addicts show increased tolerance to scopolamine. Myers⁴ habituated dogs to morphine, 10-30 mg per kilogram, and found that they showed marked tolerance to heroine and codeine as regards effect on the respiratory center, slight tolerance to the same drugs as regards the tissues governing equilibrium, and negligible tolerance as regards heart and intestinal peristalsis. He found no evidence of cross tolerance to chemically dissimilar drugs, cannabis indica and chloral hydrate. Biberfeld⁵ concludes that the habituation to morphine obtained through long-continued injections is specific, it does not exist even against heroine. In our opinion Biberfeld's conclusion is not justified by his protocols. He gave heroine to two normal dogs in doses of 5.9 and 26 mg per kilogram, respectively. In both, cerebral depression and ataxia occurred, which lasted seventy-five minutes after the smaller dose and more than three hours after the larger dose. A dog habituated to 147 mg of morphine per kilogram received 147 mg of heroine per kilogram. No ataxia resulted and only very slight cerebral depression. Another dog habituated to 97 mg of morphine per kilogram received

35.7 mg of heroine per kilogram. Brief ataxia and slight cerebral depression were noted. It is upon these results that Biberfeld bases his conclusion. In other experiments this author shows definitely that dogs habituated to morphine react to scopolamine alone or in combination with morphine in exactly the same way as normal dogs.

CONCLUSIONS

1 Dogs habituated to the daily subcutaneous injection of morphine, 150 mg per kilogram, showed tolerance to codeine and heroine.

2 Dogs habituated to the daily subcutaneous injection of morphine, 150 mg per kilogram, did not show tolerance to scopolamine.

We are indebted to Mr J. P. Quigley for assistance in making the observations recorded in this report.

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LABORATORY METHODS

SOME NEW AND IMPROVED TESTS FOR MORPHINE AND RELATED ALKALOIDS*

BY CHARLES C FULTON, OMAHA, NEBRASKA

IN THE following articles some new tests for morphine are described, and some newly discovered features of older tests, or improved methods of performing them. There are, to be sure, plenty of good tests for morphine already well known. It is one of the easiest of all alkaloids to identify. Yet some of these new tests should prove desirable additions to any list of the qualitative reactions of morphine. Attention is directed particularly to the *modified iodic-acid test*, the reaction with *nitric acid formaldehyde reagent "A,"* and the *derivative-oxidation test*. These especially are useful both for routine identification work (as in the Laboratories of the Prohibition Bureau) and for the more difficult task of identifying suspected morphine isolated from complex mixtures in a search for drugs or poisons.

ALKALOIDS USED

The reactions described in the following articles are concerned almost exclusively with morphine and the closely related alkaloids, heroin, codeine, and diionine. There are also numerous references to the reactions of apomorphine. The alkaloids used in investigating these reactions, with the source of each, were as follows:

Morphine hydrochloride—from samples

Morphine sulphate—Mallinckrodt

Diacetyl-morphine hydrochloride—Mallinckrodt

(Heroin hydrochloride)

Codeine sulphate—Mallinckrodt

Codeine (free alkaloid)—New York Quinine and Chemical Works

Dionine hydrochloride—Merck

(Ethyl-morphine hydrochloride)

Apomorphine—in $\frac{1}{60}$ gram tablets

Both the sulphate and the hydrochloride of morphine, the sulphate of codeine and the free alkaloid, were used in studying these tests. In addition, the new tests described in Articles I, II, and V have been tested on free crystalline morphine (prepared from the hydrochloride), and on morphine and codeine which I extracted from opium.

In connection with the new reagents described in Article II, the following other opium alkaloids were used:

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Narcine—Merck

Narcotine—Eimer and Amend

Papaverine—Merck

Thebaine—Merck

Oxymorphone—prepared from the morphine hydrochloride by oxidation with permanganate

These other opium alkaloids, except oxymorphone were also tried with the tests of Articles I and V. I did not feel sure that the oxymorphone was entirely free from impurities.

The unrelated alkaloids atropine, hyoscyamine, scopolamine, emetine, quinine and strychnine were also tried with the tests of Articles I, II, and V, but no effects of any importance were discovered. The unrelated alkaloids gave little or no color with the reagents described in Article II.

ACID USED

Numerous references are made in the articles to "pure concentrated" sulphuric acid, so that it is necessary to define these terms. "C. P." sulphuric acid was used, manufactured by the General Chemical Company. The analysis on the label was as follows:

Specific gravity	— 1.84
Acidity	— 95% H_2SO_4
Fe	— 0.00001%
HCl	— Nil"
As	— 0.0000005%
Nitrogen	— Nil""
Nonvolatile	— 0.0009%

1. MODIFIED IODIC ACID TEST

The iodic acid ammonia test is well known. The morphine in aqueous solution is oxidized by the addition of a few drops of iodic acid solution. This produces, unless the morphine solution is very dilute, a yellow to yellow brown color, not all of which is due to the iodine set free. Addition of ammonium hydroxide causes the color to become dark mahogany brown. This test is said to be very distinctive (Fuller, *Chemistry and Analysis of Drugs and Medicines*).

The modification consists in adding a few drops of hydrogen peroxide solution before the ammonia is added. Then instead of dark brown a bright red color is produced. This red color appears more quickly than the brown but gradually fades. The test is sensitive and is probably just as characteristic in this form as the unmodified iodic acid ammonia test. It may be applied directly to a fairly dilute aqueous extract of opium as there is no difficulty in recognizing the red color in such a light brown solution.

Removal of the free iodine by shaking with chloroform or carbon tetrachloride, before adding the ammonia, does not affect the reaction. The red color is not extracted by any immiscible solvent thus far tried. Other oxidizing agents may be used instead of iodic acid, but no other has been found that gives quite as good results. Codeine does not give the test.

II NEW REAGENTS FOR THE OPIUM ALKALOIDS

If, in testing morphine, the nitric acid test and the sulphuric-acid formaldehyde test are performed on adjacent spots of a spot plate, the nitric acid test being performed first, it often happens that the fumes from the concentrated nitric acid so affect the morphine on the adjacent spot that blue and green colors are obtained with the Marquis' reagent (sulphuric acid and formaldehyde), in addition to the usual red-purple color.

Now it is easily possible to prepare a reagent, using nitric acid, formaldehyde, and sulphuric acid, which will give blue and green colors with morphine and closely related alkaloids, practically to the exclusion of the usual purple colors given with Marquis' reagent.

Put 1 drop of concentrated HNO_3 in a dry test tube and add 4 or 5 drops of 40 per cent formaldehyde solution and then about 10 cc of concentrated H_2SO_4 . A violent reaction takes place as the first H_2SO_4 is added. When the effervescence has subsided, the reagent is ready for use. The formaldehyde must be in excess of the HNO_3 , and may be considerably in excess without greatly changing the reagent. The exact amount of H_2SO_4 used is not material.

This reagent gives with morphine a momentary purple color, quickly blue green, changing gradually to dark blue, and finally becoming dark, dirty purplish-brown-red. Codeine gives olive, changing to bright green, which gradually becomes very dark green. These blue and green colors are quite intense.

This reagent will not keep. The first noticeable changes are that morphine gives a momentary brownish instead of a purple color, and blue colors appear in the codeine reaction. If simply allowed to stand in the test tube, the reagent decays to uselessness in something like a week, but if kept in a glass-stoppered pycnometer bottle and a drop of 40 per cent formaldehyde solution added each day that it is to be used, it may be preserved in practically its original condition for a long time.

This reagent in its decay passes through a well-marked stage in which the reactions given by morphine and codeine are entirely different. It is not difficult to obtain this stage in a few minutes by dilution with water, consequently we have here a second reagent which may be of value in some cases. The reagent already described may be called Reagent A, and this second one, prepared from it by dilution, may be called Reagent B.

It is somewhat difficult to prepare Reagent B with uniform success, and it should always be tested on known morphine and codeine (both) before being tried on an unknown. However, the following procedure should give good results at least four times out of five. Put 1 drop concentrated HNO_3 in a dry test tube, follow with 3 drops 40 per cent formaldehyde solution and 1 cc concentrated H_2SO_4 . Cool in running water for a moment, add 1 cc water, cool, then add 4 cc concentrated H_2SO_4 and mix thoroughly, cooling with running water. This reagent then should give with morphine a brown color, changing gradually to dull purplish or violaceous red, and with codeine an olive-brown, changing to olive-blue-green and gradually to deep bright blue, becoming slowly purple-blue.

REACTIONS OF THE OPIUM ALKALOIDS WITH THE NITRIC ACID FORMALDEHYDE REAGENTS

REAGENT A

Morphine Momentary purple, quickly blue green gradually dark blue, and finally dirty purplish brown red
Codeine olive changing to bright green gradually very dark green
Heroin like morphine
Dionine like codeine
Apomorphine (fragment of 1/20 grain tablet) purple changing to dull dark green finally brownish red purple
Oxydimorphine olive green changing to strong bright bluish green
Papaverine the solid turns purple brown before dissolving, on solution there is little color at first but a deep bright blue soon develops
Narcotine yellow before dissolving then dirty violet red changing through brown to bright red
Narceine dark yellowish brown changing to orange, then red, then brown (about the same as with Marquis Reagent)
Thebaine red brown before dissolving then bright orange red changing to brownish orange (about the same as with pure concentrated H_2SO_4)

REAGENT B

Morphine brown changing gradually to dull purplish or violaceous red
Codeine olive brown changing to olive blue green and gradually to deep bright blue becoming slowly purple blue
Heroin same as morphine
Dionine same as codeine
Apomorphine (fragment of 1/20 grain tablet) dirty green changing to blue and slowly becoming purple
Oxydimorphine brown, changing slowly to light blue green then to green
Papaverine slight gray gradually deep bright blue
Narcotine yellowish changing gradually to bright carmine red
Narceine yellow brown, then with a greenish tinge then slightly purplish at edge brownish solution finally brownish orange (Colors not strong, not distinctive)
Thebaine bright orange red

III PELLAGRI'S TEST

Pellagri's test for morphine consists in converting it to apomorphine and detecting the latter by the strong bright colors of its oxidation product. This is a well known test which is discussed here because of its relation to the "derivative oxidation test," described under V, following.

The directions usually given for Pellagri's test (for instance by Autenrieth Warren *The Detection of Poisons*) are as follows. Dissolve a little morphine in about one half c.c. of concentrated HCl and add a drop or two of concentrated H_2SO_4 . Evaporate off the HCl at from 100° to 110° C. on the water bath. Take up the H_2SO_4 residue with water, add $NaHCO_3$ in excess and oxidize with a drop or two of iodine in alcohol avoiding excess. The aqueous solution is emerald green, and ether extracts pink or red.

A method which is much more convenient and better in every way is as follows. Put a little dry morphine in a test tube and dissolve in a small amount of syrupy phosphoric acid (85 per cent H_3PO_4). Heat to boiling over a small free flame. Cool, dilute with a few c.c. water and oxidize by adding several drops of HIO_3 or KIO_3 solution (5 per cent). An orange red color is produced. Add considerable ammonium acetate and the color changes through olive to bright green or blue green. Ether and other organic solvents then extract the colors characteristic of the oxidation product of apomorphine. The advantage of NH_4Ac over $NaHCO_3$ is that the former does not cause foaming when it is added to the acid solution.

IV DERIVATIVES FORMED ON HEATING IN PURE CONCENTRATED SULPHURIC ACID

Morphine—When morphine is dissolved in concentrated sulphuric acid, it begins at once to undergo an essential change. This change is made complete in a few minutes by warming the solution to about 40°C . The sulphuric acid solution remains colorless, but various tests show that the morphine has been completely converted to some entirely different compound. This new substance has the following properties:

a Not very soluble in cold water or cold dilute acid. On diluting the concentrated H_2SO_4 solution with say 10 times as much water (or more), and allowing it to stand, the compound crystallizes out as white transparent needles or rods, or small rods with rounded ends, often in rosettes. Very soluble in alkalies; soluble in concentrated H_2SO_4 , may be recrystallized from hot water or hot dilute acid.

b Does not give precipitates from the dilute acid solution (tested before the compound has crystallized out) with such general alkaloidal reagents as Wagner's, Mayer's, phosphomolybdic acid, etc. Thus it appears that the morphine has been converted into some substance which is not even an alkaloid.

c Shows close similarity to apomorphine in its color reactions. In fact, these are for the most part identical, concentrated HNO_3 , for instance, gives exactly the same succession of colors as with apomorphine: red-purple to red to orange. But there are some differences, notably in regard to the different solubilities of the oxidation product. (See derivative-oxidation test.)

d Like apomorphine a strong reducing agent, more easily oxidized than morphine.

Of course the resemblance of the color reactions of this compound to those of apomorphine is not accidental. The two must be nearly alike in constitution. It may be that the only difference is one of polymerization.

It will be noted that the sulphuric acid solution is to be heated only to about 40°C to produce the compound under discussion. If it is heated too hot, say to 100° , the derivative will not crystallize out as before, and various color reactions show that there has been a further change. A study of the color reactions shows that this further change takes place at from 60° to 70°C . For instance, the color reactions obtained by adding a tiny crystal of KNO_3 , or a few grains of NH_4 persulphate, to the H_2SO_4 solution, and the amount of color extracted from the green solution of the oxidation product by amyl alcohol, all show a change at about the same point. Table I shows the observed results.

TABLE I

TEMP. $^{\circ}\text{C}$	H_2SO_4 SOLUTION		AMYL ALCOHOL EXTRACT
	KNO_3	NH_4 PERSULPHATE	
20°	orange red to purple to gray blue to green	olive to green	good blue
30°	"	"	strong blue
40°	"	green	"
50°	"	"	
60°	orange red to purple to purple brown	olive to yellow brown	good blue
70°	orange red to dull purplish red to purplish red brown	"	weak blue
80°	intense orange red to dark red	orange brown	faint blue
90°	"	"	no color
100°	"	"	"

From 100° to about 140° C the reactions are practically unchanged. At the latter temperature or somewhat above it, a dark olive green color begins to develop in the concentrated sulphuric acid solution. This sulphuric acid color test merits separate discussion.

That morphine is converted into a compound resembling apomorphine in its color reactions when heated in concentrated sulphuric acid has, of course, long been known. Husemann's test is the application generally given. The degree of heat necessary has been greatly exaggerated, however, and so far as I have been able to learn, the production of a crystalline derivative insoluble in cold water or dilute acid on heating only to 40° C has been entirely overlooked. The derivative oxidation test described in the following article also seems to be new, and certain other tests easily made on this morphine derivative, have been given little attention, notably the derivative iron test.

Codaine.—When codeine is warmed in concentrated sulphuric acid it is converted into a different substance in the same manner as morphine. The two derivatives are not the same. The codeine derivative retains its distinctive qualities until the sulphuric acid solution in which it is formed has been heated at least to 100° C.

On dissolving codeine in concentrated sulphuric acid and warming to 40°, or at the most, 50° C a crystalline derivative is formed having the following properties:

a Not very soluble in cold water or cold dilute acid, soluble in alkali or concentrated H_2SO_4 . Crystallizes out as transparent white needles or rods in sheaves, rosettes, or spheres. Sometimes with considerable codeine soon after diluting the H_2SO_4 solution with water the whole aqueous solution sets to a white jelly, in which the crystals gradually form as spheres of needles like little cockle burs, the jelly gradually disappearing.

b Like the corresponding morphine derivative, it does *not* give precipitates with the general alkaloidal reagents from the dilute acid solution.

c Color reactions similar to those of apomorphine, and more closely similar to those of the corresponding morphine derivative, but in some reactions distinctly different from either. Thus, this codeine derivative is like the morphine derivative and different from apomorphine in the solubilities of the colored oxidation product, and is different from both in not giving any color reaction in the iron test.

d A good reducing agent, though codeine itself is not considered a reducing agent at all. But it is not so easily oxidized as the corresponding morphine derivative, and the latter not so easily as apomorphine.

With codeine, as with morphine, a further change takes place at about 60° to 70° C as shown by the color reactions. The change is of the same nature as with morphine.

At about 100° C on heating in concentrated sulphuric acid the codeine derivative begins to lose the peculiarities which distinguish it from the corresponding morphine derivative.

At from 140° to 150° C, as with morphine, a dark olive or olive green color develops, and the same sulphuric acid color test is obtained.

Heroin—So far as I have discovered, heroin reacts exactly like morphine when heated in pure concentrated sulphuric acid. This is natural, because of the ease with which heroin is hydrolyzed to morphine.

Dionine—The dionine reaction is practically the same as that of codeine. Such differences as there are show that dionine is intermediate between codeine and morphine in its chemical character. Thus, dionine begins to give the iron test after heating to only about 70° in concentrated sulphuric acid, while codeine requires 100°. This fact may be used conveniently to distinguish between dionine and codeine. The test will be described in the next article.

EXACTNESS OF THE TEMPERATURES STATED

Many, perhaps all, of the changes produced immediately at a certain temperature are effected gradually at lower temperatures. When morphine is dissolved in concentrated sulphuric acid and the solution allowed to stand overnight (as in some directions for Husemann's test), it is changed more than when heated within a few minutes to from 40° to 50°. In following procedures given in this and subsequent articles, the test tube containing the alkaloid freshly dissolved in concentrated sulphuric acid is to be placed in a suitable bath and heated steadily, with sufficient rapidity or slowness that the contents of the tube will be heated *to*, rather than *at*, the temperature shown by the thermometer in the bath.

COMPARISON OF COLOR REACTIONS

In Table II a comparison is given between the color reactions of apomorphine and those of the morphine and codeine derivatives, formed by heating in pure sulphuric acid to from 40° to 50° C.

Eidman's reagent does not give strong, well-marked colors, the reactions described for this reagent are not to be considered very reliable.

TABLE II

	APOMORPHINE	MORPHINE DERIVATIVE (HEROINE DERIV SAME)	CODEINE DERIVATIVE (DIONINE DERIV PRACTICALLY THE SAME)
Concentrated nitric acid	red purple changing to red and then to orange	red purple changing to red and then to orange	red purple changing to red and then to orange
Froehde's reagent	bright green	bright green	bright green
Marquis' reagent	purple changing to green black	purple changing to green black	yellowish, brownish, then deep green develops
Erdman's reagent	green changing to purple	purple changing to green blue, finally dull green	orange changing to blue green then to dull blue
NH ₄ persulphate to H ₂ SO ₄ sol	develops olive brown changing to dull green	develops yellowish green changing to green	develops bluish green changing to green
Oxidation test	orange red in acid sol, green in neutral or slightly basic sol, amyl alc ext blue, ether purplish red, chloroform purple	orange red in acid sol, green in neutral or slightly basic sol, amyl alc ext blue, ether and chloroform ext no color	orange red in acid sol, green in neutral or slightly basic sol, amyl alc ext blue, ether and chloroform ext no color
90° to 100°	NH ₄ Ac—light purplish blue, Na ₂ CO ₃ —red	NH ₄ Ac—light purplish blue, Na ₂ CO ₃ —red	no reaction

V THE DERIVATIVE OXIDATION AND DERIVATIVE IRON TESTS

The "derivative oxidation test" and the 'derivative iron test' may be conveniently performed at the same time. Both depend on the fact that morphine heated in concentrated sulphuric acid to from 40 to 50° C changes into a compound giving color reactions similar to those of apomorphine.

Procedure—Put a little morphine or its salt in a clean dry test tube and dissolve it in from 12 to 16 drops of concentrated H_2SO_4 . Stand the tube in a beaker of water with a thermometer and warm to from 40° to 50° C (not over 60° C). Remove tube and add 3 or 4 c.c. of water. Divide the solution into two portions for the 2 tests.

1 Derivative Oxidation Test This is given by morphine, heroin, codeine, and diionine. Add 3 or 4 drops 5 per cent HIO_3 solution. An orange red color develops (Bright red with considerable morphine, light orange with very little). The solution may be heated to hasten the oxidation. Heating it to boiling will do no harm and ensures complete oxidation. However it is scarcely necessary to heat with morphine or heroin, but heating is advisable with codeine or diionine. Add considerable NH_4Ac or an excess of NaHCO_3 . The latter is preferable except for the foaming it causes, which is sometimes very troublesome. The color changes to bright green or bluish green. If a larger volume of solution is desired additional water may be added after oxidation, either before or after destruction of the excess of acid. The solution to which the HIO_3 is added should contain about 4 drops of concentrated H_2SO_4 per c.c. particularly for oxidation of the codeine derivative. The derivative of morphine is more readily oxidized. Shake the green solution with a little amyl alcohol. Most of the color or even all of it goes into the amyl alcohol, which becomes blue.

2 Derivative Iron Test This test is given by morphine and heroin but not (at the temperature stated) by diionine or codeine. Narcotine reacts to the test, though not quite the same as morphine. Add 1 or 2 drops of quite dilute FeCl_3 solution (Say about 0.2 per cent. Dilute 1 drop of 10 per cent FeCl_3 with 1 c.c. of water for this purpose). Or 1 drop of FeSO_4 solution may be used instead. Add Na_2CO_3 in excess. Just as the excess of acid is destroyed, a blue color suddenly develops, changing at once to purple and then with the excess of Na_2CO_3 it becomes and remains red.

A few additional facts about each of these tests may now be given.

DERIVATIVE OXIDATION TEST

This is believed to be an entirely new test but its resemblance to Pella gri's is obvious. The green color in practically neutral or slightly alkaline solution and the blue in amyl alcohol are the same. In this test however, ether or chloroform cannot be substituted for amyl alcohol, as neither one extracts any color at all. (Except that due to the iodine set free, not noticeable with such quantities of morphine as will ordinarily be used for this test). In fact, the only organic solvents thus far discovered which will dissolve out this green color are the lower alcohols and acetone. Isopropyl alcohol and acetone extract the color from a solution saturated with salt. Ethyl and methyl alcohols used in conjunction with chloroform, also extract it from a satu-

rated salt solution, and normal butyl alcohol extracts it. These solvents all become blue. Caprylic alcohol does not extract the color.

This oxidation product is therefore sharply distinguished from the oxidation product of apomorphine by its failure to dissolve in most organic solvents. The oxidized apomorphine dissolves in all kinds of organic solvents with red, blue, purple, and violet colors. This other oxidation product of dehydrated morphine shows only blue and green colors, but as the oxidized apomorphine is also blue in the lower alcohols, the only actual difference of color is in acetone. In this solvent the apomorphine oxidation-product is blue with a distinct purple tinge, while the other is at first simply blue, then greenish-blue, and on standing turns to green and then to yellowish.

As already mentioned, the codeine-derivative is not so easily oxidized as the morphine-derivative, and the latter not so easily as apomorphine. This is shown by the relative ease with which iodic acid produces an orange-red color in a solution only slightly acid. The diionine-derivative is in between those of codeine and morphine, heroin reacts just like morphine. The derivatives of morphine, codeine, and diionine (formed by warming in concentrated sulphuric acid) seem to give the same oxidation product.

The oxidation product of apomorphine is less easily reduced than the other. The color change of the oxidized morphine derivative in acetone has already been described. This is doubtless due to reduction. The oxidized apomorphine shows a similar change when treated with a strong reducing agent. For instance, on treating its blue solution in amyl alcohol or its red solution in ether, with a drop of stannous chloride solution, the color is changed to green and then to light yellow or colorless. (Wangerin's test.)

If to the green solution of the oxidized morphine-derivative considerable concentrated ammonia is added, the color is changed gradually to purplish blue. Amyl alcohol readily extracts all the color from this solution, and becomes purple.

If the solution of the alkaloid in concentrated sulphuric acid is heated too hot, say to 100°C , the orange-red color is produced on oxidation and the green on subsequent neutralization, just as described for the test, but neither amyl alcohol nor any other immiscible solvent thus far tried will then extract any color at all.

DERIVATIVE-IRON TEST

This test is not new, though it has scarcely been described in the form given here. (See Perkin *Qualitative Chemical Analysis*.)

If NaHCO_3 is substituted for Na_2CO_3 , the final color is a purplish-pink. NH_4Ac gives light purplish-blue. NH_4OH gives sometimes violet and sometimes red, or an intermediate color. These colors are not extracted by amyl alcohol nor by other immiscible solvents. If considerable NH_4OH is added and the solution is allowed to stand overnight, the color is then more of an orange-brown by transmitted light, and on looking obliquely down the tube a strong and beautiful purple fluorescence is seen.

The ferric chloride may be added either before or after the excess of acid is destroyed. If the carbonate or bicarbonate is added first, a slight pink color

is generally produced, due to a trace of iron in the reagent. The reaction may be used as a sensitive test for iron in the sulphuric acid, the water, or the substance used to destroy the acidity. In fact, an excessive amount of iron in the magnesium oxide of this laboratory was discovered through experimenting with this test.

Distinguishing Dionine from Codeine—This iron test provides a simple and convenient means of distinguishing dionine from codeine. The latter gives the test only after heating in concentrated sulphuric acid to above 100°C , while dionine reacts after heating to above 70° or 75° , and may even give a very faint test after heating only to 60° or 50° . Dissolve the alkaloid or its salt in a little concentrated H_2SO_4 , heat to 80° or 90°C , dilute, add 1 or 2 drops of dilute FeCl_3 and Na_2CO_3 in excess. If a good red color is obtained, the alkaloid is dionine; if the color is only light yellowish or brownish, it is codeine. By testing suspected dionine in comparison with known codeine, or vice versa it would scarcely be possible to make a mistake. So far as morphine and heroin are concerned, practically the same test is obtained after heating in concentrated H_2SO_4 to anywhere from 100° to 140°C .

Reaction of Narcotine—Narcotine also changes its properties when warmed in concentrated sulphuric acid, the change being probably one of hydrolysis rather than dehydration as with morphine. One of the new properties is that of giving a reaction in the iron test. With sodium carbonate a brownish red color is obtained which soon resolves itself into a reddish brown precipitate and a light pink solution. Ammonium acetate gives a light grayish blue color.

The Iron Test in Conjunction With Pellagri's Test—Apomorphine, without any heating in acid gives the colors we have described for the iron test. Solvents may extract some of the color, however. In particular, amyl alcohol extracts the color from the ammoniacal solution, becoming brownish purple. So the iron test may be used along with Pellagri's test, as well as with the derivative oxidation test. Such use is less convenient, however. With only a moment's boiling in phosphoric acid to produce the apomorphine, it will be found that morphine generally gives the iron test quite well, dionine faintly, and codeine not at all, while all give Pellagri's test quite well. Thus, it appears that apocodeine does exist and if it is a mixture, it does not necessarily contain apomorphine as has been suggested. With longer boiling in the phosphoric acid the apocodeine is broken down, the solution gives the iron test and no doubt does contain apomorphine.

VI HUSEMANN'S TEST

All authorities give Husemann's test as one of the most important tests for morphine, but practically every one gives a method for performing it somewhat different from that given by any of the others and a somewhat different description of the colors produced. (Consult, for instance Fuller, *Chemistry and Analysis of Drugs and Medicines*, Antenrieth Warren, *The Detection of Poisons*, Mulliken, *The Identification of Pure Organic Compounds*, Allen's *Commercial Organic Analysis*, etc.) A generalized description

would seem to be as follows Dissolve the morphine in concentrated sulphuric acid and heat this solution at about 100°C for half an hour Cool, and oxidize with a trace of nitric acid, or a small crystal of potassium nitrate or chlorate A momentary purple or "violet" color is produced, changing to red and then to orange (i.e., the same succession of colors as is given by apomorphine with concentrated nitric acid)

The test works all right, except that the momentary purple or violet color is seldom, if ever, obtained A blood red or dark red color is produced at once, when the oxidizing agent is added, unless the sulphuric acid is first diluted slightly If a drop of water is added to 6 or 8 drops of the sulphuric acid solution, and then a small crystal of potassium nitrate stirred in, the purple-red-orange succession of colors may be obtained The best description of Husemann's test that I have found is that given by Fresenius-Wells in their *Qualitative Chemical Analysis*, it suggests (to my mind) that the "concentrated sulphuric acid" formerly used was weaker than that in use today Confusion may have arisen, however, through misuse of the word "violet," a color-term much abused in chemical literature The solution of the oxidation product of apomorphine in ether is sometimes spoken of as "violet" or "reddish-violet," though it is simply pink when dilute, and nearly crimson when concentrated

Heating the sulphuric acid solution for half an hour at 100° discolors it more or less, if fairly well exposed to the air it becomes dirty violet Since morphine dissolved in concentrated sulphuric acid is completely changed to a compound giving color reactions similar to those of apomorphine by merely warming to 40° , most of this heating would seem to be unnecessary, and even unwise, especially if impurities are present There are, however, differences in the reaction if the solution is heated only to 40° rather than to 100° , but the 40° reaction is probably just as important and interesting as the other, heating to 100° suffices for the other, and this may be done in five or ten minutes just as well as in half an hour Incidentally, the reactions are more like those of apomorphine after heating only to 40°

A test will now be described which includes several reactions making use of the sulphuric acid solution (containing the derivative of morphine) after heat treatment The effects of ammonium persulphate and formaldehyde are included here for convenience, though the persulphate gives a different sort of oxidation from the nitrate or the chlorate, and the formaldehyde reaction is of course entirely different

Dissolve a small amount of dry morphine or its salt in about 1 c.c. of pure concentrated H_2SO_4 , place the test tube in a beaker of water with a thermometer, and heat to 40° or 50°C Draw out about half the acid, putting from 6 to 10 drops on each of five spots of the spot plate Continue heating the remaining acid solution

The acid solution which has been heated to 40° or 50° gives the following reactions

HNO_3 (streak) Dip a glass thread in concentrated HNO_3 and draw it through the solution Its path is marked by a blood-red streak, the streak being first strong orange-red, then *blood-red*, and then dark red Meanwhile

the solution near the streak becomes orange red changing to red, and the outer parts of the solution become first *purplish* and then bluish

HNO_3 (trace) Add a very small trace of concentrated HNO_3 and stir it in. An orange red color is produced which changes at once to purple and this soon to gray blue and then to dull green. A tiny crystal of KNO_3 gives the same result. Excess of KNO_3 or HNO_3 must be carefully avoided, in order to get the succession of colors stated. Otherwise the solution will probably become and remain red or orange red.

KClO_3 Add a very tiny crystal of KClO_3 to the solution, crush, and stir it in. The crystal turns orange when added, as it is stirred in the solution becomes *purple*, this quickly changes to gray blue and then to *green*. If too much KClO_3 is used, orange and brown are the final colors produced though the purple is generally noticed easily.

$(\text{NH}_4)_2\text{S}_2\text{O}_8$ Stir in a few grains of ammonium persulphate. An olive green or *green* color soon develops.

Formaldehyde Add a trace of HCHO and stir in. A strong deep *purple* develops, changing to blue black and then to green black. (Like apomorphine.) The codeine and diionine derivatives, however, do not give purple but develop a green color.

Heat the remaining acid solution to the temperature of boiling water, then distribute it on a spot plate. With the agents already mentioned the reactions now obtained are as follows:

HNO_3 (streak) Streak *blood red* changing to *dark red*. The outer parts of the solution become *orange*.

HNO_3 (trace) Intense *orange red* changing to *dark red*.

KClO_3 Orange red to red.

NH_4 persulphate Orange brown.

Formaldehyde Dark purple brown.

With the exception of the formaldehyde reaction where the difference has already been noted, morphine, heroin, codeine and diionine all give practically the same colors in the tests.

On the whole it would seem that Husemann's test has been overrated. The derivative oxidation test is probably better even when the similar Pellagri's test is also used.

SUMMARY

1. A new test for morphine not given by codeine is described. It is a modification of the iodic acid ammonia test, and might be called the "iodic acid peroxide test."

2. Methods for making two new reagents for the opium alkaloids are given, and their reactions with the opium alkaloids described. These reagents are made from nitric acid and formaldehyde in solution in concentrated sulphuric acid, the formaldehyde being in excess of the nitric acid.

3. An improved method for performing the well known Pellagri's test is described.

4. Preparation of a new derivative of morphine is described. Codeine and diionine give similar but distinct derivatives. The derivative is formed

by heating the alkaloid in concentrated sulphuric acid solution to 40° or 50° C, and is no doubt a dehydration product. On diluting with water it then gradually crystallizes out. The morphine-derivative is not apomorphine, but it gives similar color reactions.

5 Two tests based on the formation of the derivative mentioned in the preceding article are described in detail. The derivative-oxidation test is given by morphine, heroin, codeine, and diionine, and is a new test similar to Pellagri's. The derivative-iron test has been previously described, though scarcely in the form given here. It is given by morphine and heroin, but not (after heating to only 40° or 50°) by codeine or diionine. However, the test can be used to distinguish between codeine and diionine, for after heating to 80° or 90° C it is given by diionine but not by codeine.

6 Husemann's test is discussed, with particular attention to the difference in the reactions when the sulphuric acid solution is heated only to 40° or 50° C instead of to 100°.

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- ⁹Perkin. Qualitative Chemical Analysis, 1905

A NOTE ON THE NORMAL SERUM CALCIUM CONTENT OF MAN*

BY JOSEPH H. ROE, PH.D., AND BERNARD S. KAHN, M.D., WASHINGTON, D. C.

THE generally accepted normal blood calcium content for a man of 9 to 11 mg per 100 cc of serum is based upon reports in practically all of which the Kramer-Tisdall¹ permanganate titration method was used. However, Rosen and Krasnow² recently reported values obtained by the latter technique which were definitely higher than the usually accepted normal. These authors found values of 10.7 to 13.2 mg per 100 cc of serum in a series of analyses of sera from 50 medical students. As other controversial reports are in the literature concerning what is the normal value for blood calcium, it occurred to us that it would be of interest to carry out a series of serum calcium determinations upon normal subjects, using the colorimetric method of analysis we³ have developed.

In our method the proteins of the serum are removed by adding 4 volumes of 10 per cent trichloroacetic acid. Five cc of the trichloroacetic acid filtrate is alkalinized until distinctly pink to phenolphthalein, 1 cc of 1 per cent Na_3PO_4 solution is added and, after one hour's standing, the mixture is

*From the Department of Biochemistry, George Washington University Medical School.
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centrifuged and the supernatant fluid is decanted. The mat of $\text{Ca}_3(\text{PO}_4)_2$ in the bottom of the centrifuge tube is washed twice with 50 per cent alcohol of N/0.05 alkalinity, centrifuging two minutes to throw down the $\text{Ca}_3(\text{PO}_4)_2$ in each instance. The $\text{Ca}_3(\text{PO}_4)_2$ is then dissolved in 10 cc of H_2SO_4 of approximately 1.4 normality. This solution and 10 cc of standard phosphate solution containing 0.05 mg of phosphorus acidified to approximately 1.4 normality with H_2SO_4 are treated with 1 cc of 5 per cent sodium molybdate and 1 cc of 0.5 per cent hydroquinone in 15 per cent sodium bisulphite. The solutions are then boiled for ten minutes, cooled and made up to a definite volume with water, usually 15 cc. The unknown solution is then compared with the standard in a colorimeter.

In this paper we are reporting a series of calcium determinations upon the sera of fifty normal subjects. These subjects were medical students and nurses with no evidences of disturbed calcium metabolism. The values obtained in this series of analyses are shown in the table. They vary from 9.0 to 11.6 mg per 100 cc of serum the average being 10.13 mg per 100 cc. These findings are in accord with the generally accepted normal of 9 to 11 mg per 100 cc of serum and are of interest in that they were obtained by a new and entirely different technique.

SERIAL NO	MG OF CALCIUM PER 100 CC OF SERUM	SERIAL NO	MG OF CALCIUM PER 100 CC OF SERUM
1	10.1	26	10.1
2	9.2	27	11.4
3	10.4	28	11.6
4	9.7	29	9.7
5	9.3	30	10.2
6	9.4	31	10.3
7	9.7	32	9.9
8	10.5	33	10.6
9	9.0	34	9.9
10	9.0	35	9.8
11	9.4	36	9.5
12	9.3	37	10.0
13	9.4	38	10.3
14	9.5	39	10.4
15	10.7	40	9.7
16	10.8	41	10.3
17	11.4	42	9.7
18	10.1	43	10.4
19	11.2	44	10.6
20	9.4	45	11.1
21	9.1	46	10.7
22	11.2	47	9.8
23	9.4	48	9.1
24	10.1	49	10.8
25	11.2	50	11.2

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 Rosen, I. and Krasnow, F. A Note on the Calcium Content of the Serum of Normal Adults. *Jour Lab and Clin Med* 1926, *xii*, 157.
²Roe, J. H. and Kahn, B. S. A Colorimetric Method for the Estimation of Blood Calcium. *Jour Biol Chem*, 1916, *lxv*, 58.

A NOTE IN CONNECTION WITH READING RESULTS OF THE KAHN PRECIPITATION TEST*

BY HERBERT SILVETTE, RICHMOND, VIRGINIA

THE commonest complaint that one hears in regard to the Kahn test is the difficulty in reading the reaction, once it is finished. The difficulty in this case is not due to the fineness or the delicacy of the floccules, for even a one-plus test is very definite as regards the precipitation, but is caused by the kind and source of the light by which the reactions are usually read.

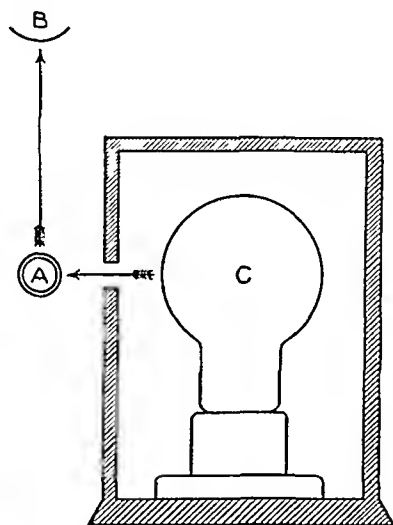


Fig 1.—Diagrammatic representation of the ultramicroscope principle applied to reading the Kahn test. *A*, reaction tube; *B*, eye; *C*, source of light. Arrows show path of light.

Reflected, not transmitted, light should be used to judge the amount of a flocculent precipitate. As illustrated by the ultramicroscope, reflected light will reveal particles in suspension much too small to be seen by any other form of illumination. When this same principle is applied to reading the results of the Kahn test, the reaction tube is illuminated by a narrow beam of light which strikes the floccules at right angles to the line of vision from the eye to the tube. The smallest amount of flocculation then seen constitutes a doubtful or positive reaction, the negative tubes are invariably opalescent when read by reflected light.

As illustrated, the reaction tube should be held within a half inch of, and directly opposite, the aperture through which the light is issuing. The eye may be at any distance above the tube, depending upon its individual focus,

*From the Pathological Laboratory, Johnston-Willis Hospital.
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but in order to get the most perfect results the beam of light and the line of vision must be perpendicular to each other. In explanation of this the light passes horizontally through the liquid and is reflected by the floccules in suspension, if present, vertically upward to the eye. Should there be no precipitate, no light is reflected, it passes in a straight line directly through the tube.

When the apparatus described is used in a dark room so that a single beam of light is all that illuminates the tube, results of the Kahn test may be reported with the fullest confidence that in so far as the reading of the reactions goes neither a false positive nor a false negative was obtained.

THE ISOLATION OF YEASTS AND MOLDS*

By GRACE A. HILL, A. B. M. S. BELMONT, CALIFORNIA

WE HAVE found a very satisfactory medium for the isolation of yeasts and molds in the Stritz¹ modification of Lund² wort agar. Stritz substitutes "near beer" for the wort because it is more easily obtainable and gives quite as good results.

Near beer agar has the advantage of being simple to prepare. The contents of one pint bottle of near beer is made up to 900 cc with tap water. Fourteen grams of agar are added, and dissolved by boiling. It is then filtered, flaked in 100 cc or other known portions and autoclaved. Before pouring the plates, 4 cc of sterile 5 per cent lactic acid is added to each 100 cc of melted agar.

The limited protein as well as the acid reaction serves effectually to inhibit all bacterial growth without apparently, having any effect upon the fungi.

I have used this medium with gratifying results in making yeast and mold counts of butter, and also in the examination of pathologic material in the clinical laboratory.

THE YEAST AND MOLD COUNT OF BUTTER

When the yeast and mold count of butter was first attempted in "butter standardization" at the University of California, several methods recommended by other laboratories doing this work were tried and discarded.

In making a yeast and mold count of butter any medium on which even a few bacterial colonies develop is unsatisfactory since it is difficult or impossible, to distinguish between some of the smaller celled yeasts and some of the larger bacteria, even with the low power objective which makes it necessary to fish and examine doubtful colonies in a wet mount and I believe, contrary to the popular theory, that no amount of experience will enable one

*From the Laboratory of the California Sanatorium for the Treatment of Tuberculosis. Received for publication September 16, 1927.

†It was my duty to make the yeast and mold counts of butter from January to August 1923 in the College of Agriculture, Davis, California.

A NOTE IN CONNECTION WITH READING RESULTS OF THE KAHN PRECIPITATION TEST*

BY HERBERT SILVETTE, RICHMOND, VIRGINIA

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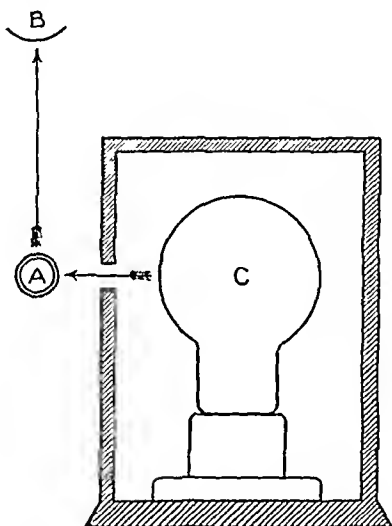


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MODIFIED WRIGHT'S TECHNIC FOR THE STANDARDIZATION OF VACCINES*

BY LELAND W. PARR, PH.D., BEIRUT, GRAND LIBAN, SYRIA

WRIGHT'S method for enumerating the bacteria present in suspensions used in the preparation of bacterial vaccines is one of the best known procedures for this purpose. Using this method¹ one draws in order, into a capillary pipette equal volumes of sodium citrate solution, normal human blood, and the bacterial emulsion to be used in the preparation of the vaccine, each of the three equal volumes separated from the other by a bubble of air. One then empties the pipette into the concavity of a hanging drop slide or into a watch glass and mixes the three substances thoroughly, usually by means of the pipette. After this smears of the mixture are made on slides for appropriate staining. If on examination the stained smears are found satisfactory, a count is made to ascertain the relative number of bacteria and red blood cells, and from this it is easy to calculate the strength of the bacterial suspension.

The method requires the speed and dexterity of an expert and the volume of the mixture so obtained is small and soon dries up if not used at once. This interferes with repeating the staining should that be desired. As an instructor of students of medicine for the past seven years, I have not yet encountered a student who could get good results with this method without an inordinate expenditure of time and materials. More recently I have been using the method described below.

First, a small amount of citrate solution is put into a serologic tube of the sort commonly used in the Wassermann test, about 0.2 cubic centimeters being a satisfactory amount to use. Then, with a capillary pipette, as large a drop of blood as possible is taken from the available normal finger or ear. Marking the level in the pipette to which this drop of blood rises it is then expelled into the citrate solution in the serologic tube, and by reaspiration all traces of the blood are washed from the pipette. Next the pipette may be washed in alcohol and ether and passed through a flame if there is any question of its sterility, after which a volume exactly equal to the volume of the blood originally taken is taken up from the bacterial suspension. The bacteria are then blown out into the blood citrate suspension, and complete homogenization is obtained by agitating the tube. I have found this to be much easier to do in this way than to obtain mixture by the usual use of the capillary pipette. When thorough mixture has been attained slides may be made from the suspension as in other methods, but it is found more satisfactory unless the blood drop was unusually large to smear the mixture on the slide with a platinum loop rather than to attempt the usual blood film.

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Kolmer Infection Immunity and Biologic Therapy, Philadelphia 1910 W. B. Saunders Co.

This method offers unusual advantages in teaching. All steps can be taken leisurely, allowing ample time for full explanation and relevant questions. The volume of the suspension is sufficient for the preparation of many slides, and it is not liable to be lost by evaporation of the diluent during an ordinary class period. For the laboratorian, also, who only occasionally makes a vaccine, the method has value, though it is not suggested that the expert would profit by changing the better known Wright's method for this modification.

A CONTAINER FOR FECES*

By E. M. WATSON, M.D., M.Sc., LONDON, CANADA

ONE of the problems which not infrequently confronts the physician or laboratory worker is the adoption of a satisfactory means whereby samples of feces may be collected, sent to the laboratory, and examined with a minimum of discomfort for all concerned. Methods have been recommended with this purpose in view. For example, Einhorn¹ has recently described a

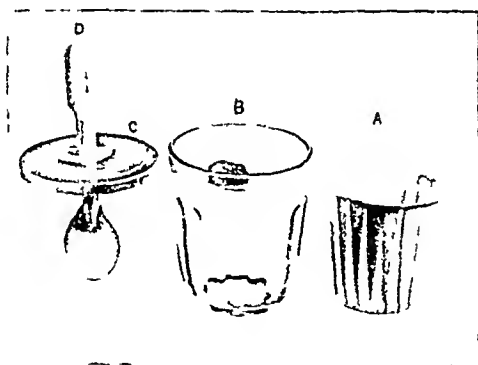


Fig. 1

container for feces which he has named the "kopioscope." It consists essentially of a glass jar with a special screw top, attached to which and projecting into the jar is a metal scoop. The advantages of such a device are obvious. There is described below a somewhat similar contrivance which embodies the principle of the "kopioscope," with certain modifications.

In Fig. 1 are shown the component parts of the utensil. A is a waxed paper drinking cup of 140 c.c. capacity. B is a 210 c.c. glass "jelly jar." C is the tin lid of the latter and through which a paper picnic spoon (D) has been passed.

*From the Laboratory of Pathological Chemistry, University of Western Ontario Medical School.

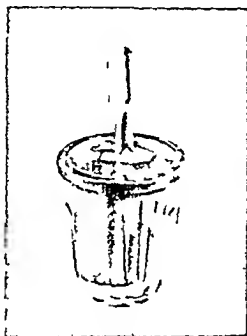
Received for publication October 1, 1927.

¹Einhorn M. Jour. Am. Med. Assn. 1927 LXXXIII 1145.

Fig 2 shows the parts assembled ready for use. The paper cup has been set within the glass jar, the lid with the spoon has been placed in position. To utilize the container, the patient or attendant removes the cover, secures a spoonful of fecal material and replaces the lid, leaving the spoon in situ.

To prevent the escape of any obnoxious odors, the small opening in the lid surrounding the handle of the spoon may be closed by means of a piece of adhesive plaster. Also, to guard against soiling the under surface of the cover, a perforated circular piece of waxed paper of the same size as the lid may be placed beneath the latter prior to collecting the sample.

After the examination of the specimen has been completed, the cup and spoon are discarded and replaced by new ones. Thus no unpleasant cleansing of soiled vessels is necessary.



Fig

It is true that the specimen cannot be viewed without removing the lid of the container as it can in the 'l oproscope' but this is not considered a very serious disadvantage in that inspection of the material is limited to the laboratory worker at the time when the other tests are carried out, and the jar does not need to be wrapped in order to prevent its contents from being exposed to general view.

The container described in this paper has been found to provide a simple, sanitary, convenient, and inexpensive utensil free from error producing influences for obtaining samples of feces as required in routine clinical practice.

The only reason for adopting the particular style of jar as shown in the photographs is that no other glassware of exactly similar pattern is in use in this hospital.

A CLINICAL TEST FOR URINARY ACETONE AND DIACETIC ACID*

BY JEANETTE ALLEN BEHRE, PH D., CINCINNATI, OHIO

DISCUSSION OF QUALITATIVE TESTS

THE various so called "tests" for acetone and diacetic acid described in the textbooks and laboratory manuals are open to criticism from at least two points of view when an attempt is made to apply them to routine urinary work. In the first place they cannot be relied upon to give satisfactory results when applied to the urine directly because of the possible presence of interfering substances, and in the second place, in each one of the tests a more intense reaction is given by one of the acetone bodies than by the other, so that it is difficult to assign any definite points at which the test may be called "positive" or "negative." In addition, some of the tests, such as the ferric chloride test for diacetic acid, are far from being sensitive enough to indicate the presence of small amounts of the substance, just above the normal, which may be very significant (1 or 2 mg per 100 cc of urine may be regarded as above the normal).

The *ferric chloride* and *iodoform* tests are both impractical for routine use, the first because it is neither sufficiently specific nor sufficiently sensitive, and the latter because even in its modified forms it is not sufficiently specific to give significant results when carried out on the urine directly.

The *sodium nitroprusside* test, which is one of the most commonly employed, is extremely sensitive to diacetic acid, when carried out with ammonia and an ammonium salt (Rotheria's test), but reacts much more slowly and less intensely with acetone. In general, it may be said that diacetic acid reacts quickly in this test, giving a deep rose to permanganate color, and that acetone gives a less intense color, which develops more slowly, but the two types of reaction grade into each other with varying concentrations of the two substances, so that it is impossible to distinguish between moderately large, and quite significant, amounts of acetone, and very small amounts of diacetic acid. Bigwood and Ladd¹ found that diacetic acid showed a positive reaction to a dilution of 1 to 20,000, but that acetone did not give color at a dilution greater than 1 to 1,000. In Cole's *Practical Physiological Chemistry*,² it is stated that with Rotheria's test acetone can just be detected in a dilution of 1 in 20,000, while aceto-acetic acid shows a positive reaction in a 1 to 400,000 dilution. By a modification of the method, using relatively large amounts of solid ammonium chloride (about 15 gm) with only about 10 drops of the solution to be tested, 1 drop of 5 per cent sodium nitroprusside and a layer of concentrated ammonium hydroxide, we have been able to detect acetone in a 0.002 per cent pure water solution, and diacetic acid at a much greater dilution. In urine, however, the faint pink color given by these small

*From the Biochemical Laboratory of the Union Central Life Insurance Co.
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amounts is usually hidden by the color of the urine itself, or the color developed from other substances. In most specimens of urine the amount of diacetic acid which yields 0.005 per cent of acetone on distillation can be easily detected, but the presence of acetone is often doubtful in the test in concentrations even higher than 0.005 per cent. The nitroprusside test is also far from specific for the acetone bodies. It is used for the detection of glutathione (Hopkins³), and in general for the presence of the SH group (Heffter⁴ and Arnold). Specimens of urine are often found which give suspicious looking colors not due to acetone bodies. Alkaline urine and urine high in urates often show such color. Formaldehyde in large amounts and some other substances used as preservatives, give interfering colors.

The *salicylic aldehyde* reaction carried out in strongly alkaline solution (Frommer's test), is extremely delicate for the detection of acetone but gives very slight color with diacetic acid. It has been found very satisfactory for use on distillates from urine or blood filtrates⁵ but cannot be used to advantage on urine (or blood filtrates) direct. This is due to the interference from sugar, which gives color under these conditions, and other substances, some of which tend to inhibit the formation of color. The fact that diacetic acid reacts to a very slight extent but not markedly is also a disadvantage.

As no test was found which gave satisfactory results when used directly on the urine, and as a distillation of all samples is impossible when large numbers are to be tested daily, the following method was devised, using the *salicylic aldehyde* reaction in an attempt to obviate these difficulties.

THE CLINICAL TEST

The reagents used are *salicylic aldehyde* and a concentrated solution of sodium hydroxide. The urine is heated in a test tube in a boiling water bath and as the vaporized acetone rises it is brought into contact with the reagents which are suspended over the mouth of the tube on a piece of cotton.

It is best to use a rather wide and short test tube (about 2 cm. in width and 12 cm. in length), but the exact dimensions are not essential. Three cc. of urine are placed in the clean test tube and one drop of 50 per cent concentrated sulphuric acid is added to facilitate the transformation of diacetic acid to acetone. A small, thin square of cotton of good quality is prepared, and one drop of pure, undissolved *salicylic aldehyde*⁶ is dropped upon the center of it. Two drops of 32 per cent sodium hydroxide are then applied over the spot of aldehyde. This is preferable to adding the hydroxide first. The two reagents solidify on the cotton to form a flat, yellow disc. The cotton is then inverted over the mouth of the test tube so that the spot formed by the reagents is turned down toward the urine. The cotton may be pushed down slightly into the tube to keep it in place, but in such a way that the disc formed by the reagents does not touch the sides of the tube. The tube is then placed in a boiling water bath for eight minutes. It is convenient to have a rack for holding the tubes upright in the bath. In the presence of

⁶There is a great difference in the intensity of color given in this reaction by different makes of *salicylic aldehyde*. The product which we have found the most satisfactory is Elmer and Amend's Acid Salicylic Synthetic (*Salicylic Aldehyde*). This reagent should be kept in a dark glass stoppered bottle. It may be added to the cotton from a dark colored dropping bottle.

of blood, *patient's cells plus donor's serum* or *patient's serum plus donor's cells*, may be mixed

It is our custom always to make a graphic diagram of the position on the plate in which each drop of blood is to be mixed, which eliminates all possibility of error and also gives a permanent record that will be of value should it ever be desirable to make a large grouping of the individuals whom we have typed under either of the two generally accepted classification systems. The blood specimens having been taken, defibrinated, and separated by centrifugation we next mix one drop of the recipient's corpuscles with four drops of the serum of each of the donors, and a drop of each of the donor's corpuscles with four drops of the serum of the recipient, with a glass stirring rod, each in its proper place on the typing plate. The plate is then easily lifted from the desk by the cylindrical handles and oscillated with a rocking motion which keeps all of the corpuscles in movement for the desired length of time. We hold the plate while oscillating over a white background, or better still

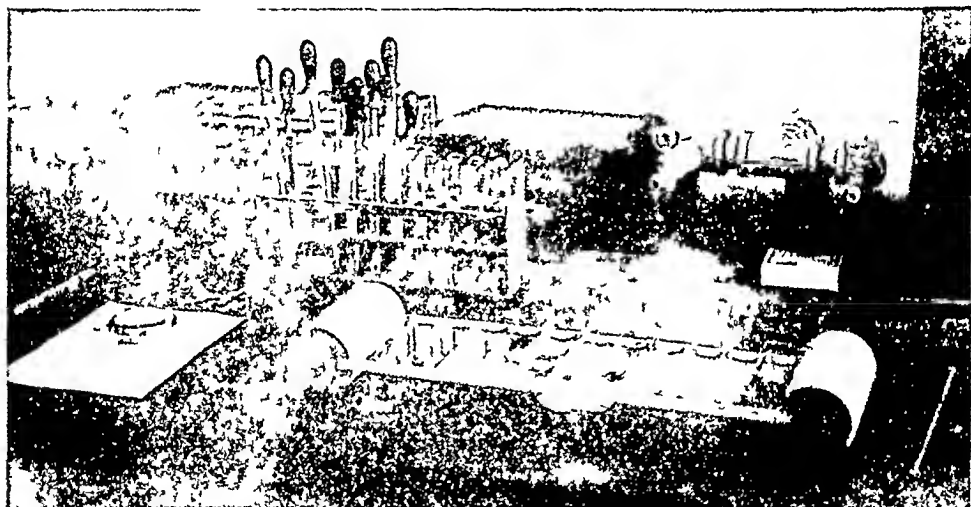


Fig 1—Author's blood typing plate as made for him by the Arthur H. Thomas Company and a corner of the serologic desk at the Laboratory of the Florence Infirmary

over a hole in the desk fitted with an electric bulb beneath as used for the microscopes, observing the drops by transmitted light for evidences of agglutination. As agglutination shows up, the donors in question may be ruled out by their number or designating initials, and the selection made at the end of the seven-minute period from among those donors whose specimens have shown no agglutination in either side of the cross-match.

SUMMARY

1 The macroscopic slide agglutination method of blood typing is considered the most sensitive and efficient method for the selection of donors for transfusions.

2 A rapid technic is described making use of a ruled glass plate by means of which a number of donors may be typed against a prospective recipient at one time.

REFERENCE

McLeod, James. The Transfusion of Blood, Jour S Carolina Med Assn., 1926, *xiii*, 57

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D. ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

Live Fishes Impacted in Food and Air Passages of Man Gudger E. W. *Arch Path and Lab Med*, September 1926, 35, 355

This data given show that on the coasts of France and Italy and in India and the Far East fishermen are accustomed to take live fishes between their teeth either to kill them by biting, or to hold them while the hands are used to free the hook or net. A fish so held if it pricks the man's mouth with its spines or sets up any sudden movement, will readily cause the involuntary opening of the mouth with a resultant entrance of the fish into the throat of the man.

In time, accounts of the impaction of live fishes in the food and air passages range from Coganus, 1567 to Jordan 1924—a range of 357 years.

Twelve countries are represented in these various accounts as follows: India leads with five cases, and France follows with three. Burma and Italy tie for third place with two cases each, Algeria, Ceylon, Germany, Japan, Philippine Islands, Scotland, and Senegal are credited with one each, and finally there is one account (Coganus' the oldest) not assignable to any country.

ARTHRITIS DEFORMANS The Role of the Dysentery Bacillus in Arthritis Deformans Clifford, S. H. *Am Jour Dis Child* July 1926, xxxii, 72

That the symptom complex called arthritis deformans of Type I may in some cases be the arthritis following an unrecognized dysentery bacillus infection is the possibility raised by the observations here reported. Seven cases of arthritis deformans are reported in four of which there was evidence of either an active or a past infection with *B. dysenteriae* Flexner.

The literature shows arthritis following a typical dysentery to be a well established clinical entity that may occur as long as four weeks after the acute attack.

It is possible to have a dysentery bacillus infection without its producing a clinical syndrome recognizable as dysentery.

Dysentery should be considered as an etiologic factor in arthritis deformans (Type I) and only ruled out after bacteriologic studies and agglutination reactions using a complete series of antigens, are proved to be negative.

Under the exceptional conditions governing these observations the dysentery reaction proved to be a specific diagnostic test. It has not been studied on a large enough scale to permit any general conclusions as to its value.

The term 'dysentery test' is applied to the intradermic injection of 0.1 cc of formalinized dysentery vaccine.

Xanthochromia of the Spinal Fluid in the Newborn Kohlbray O. *Am Jour Dis Child* July, 1926, xxxii, 58

A series of five newborn infants with xanthochromia of the spinal fluid is here presented.

The color of the fluid varied from a pale yellow to a bright canary. All these fluids showed red blood cells, either all or in part crenated when examined a few minutes after withdrawal. No difficulty was encountered with any of the spinal punctures, what might be termed "clean punctures" in that there was no evidence of

blood at the time. Some signs of intracranial disturbance were noticed before punctures were done in all these cases. These signs, in the order of their frequency, were uncontrollable vomiting, listlessness, mild cyanosis, nystagmus, and a very high temperature.

There is evidence, based on the characteristics of these fluids and the clinical course of these infants that yellow fluids, such as are here described are related to previous intracranial blood vessel derangement, producing either transudation of blood plasma, frank hemorrhage, or both. The etiologic agent may be intracranial congestion occurring during labor.

The material here presented is purely clinical. Further knowledge of this condition will be gained only as such fluids are examined more carefully, with particular reference to coagulability, albumin content, cell content, and the presence or absence of bile and hemoglobin or their derivatives.

MEASLES PROPHYLAXIS Report on the Use of Immune Goat Serum, Hayne, A. H., and Gasul, B. M. *Jour. Am. Med. Assn.*, October 16, 1926, LVIII, 1185

Tunnichiff's immune measles serum has been of definite value as a prophylactic for measles.

If the serum is administered to contacts not later than the fifth day of exposure, always counting the date of onset as the first day of the disease, protection seems assured in about 90 per cent.

Those who receive serum early, yet are not wholly protected, pass through an attenuated form of the disease.

Passive immunity endures from two to four weeks and in some instances even longer. A child was placed, through error, in a measles ward two weeks after he had received 7 cc of immune goat serum intramuscularly. This child, because of his close contact with many cases of measles in all stages of the disease, was believed to be hopelessly exposed and so was not removed from the ward, where he remained for more than two weeks. He did not contract measles.

The serum produced no deleterious effects, regardless of age or physical condition of the patients in whom it was used.

The advantages of the Tunnichiff serum, as compared with convalescent measles serum, are evident when the question of supply is considered. Ultimately, it is hoped, the Tunnichiff serum will be easily available for all who desire it, whereas a similar situation can scarcely be hoped for in respect to convalescent serum.

LIVER DAMAGE IN TREATED SYPHILIS Urobilinogen Determination in Treated Syphilitic Patients, Brown, H., and Greenbaum, S. S. *Arch. Dermat. and Syph.*, October, 1926, LV, 434

A sensitive test of liver function is of prime importance to the syphilographer because of the well known effects on the liver of commonly used antisyphilitic remedies.

The inability of damaged liver cells to remove urobilin from the portal circulation necessitates elimination by the kidneys, and therefore it has been proposed that some idea as to the presence or even the extent of hepatic damage may be had from examinations for urobilinuria.

The authors studied the question by the application of the test described by Wallace and Diamond (*Arch. Int. Med.*, June, 1925, LVV, 698) to 100 cases of syphilis under treatment. Over 500 determinations were made. Their conclusions were that the results of urobilinogen determinations on the urine of treated syphilitics were too variable to serve as an index of hepatic damage in such patients.

BLOOD SEDIMENTATION Significance of Blood Sedimentation in Urology, Litten, L., and Szpiro, J. *Ztschr. f. Urol.*, 1926, LV, 481

The authors made 118 tests on 41 patients in the Urologic Section of their hospital by Linzenmeier's method and came to the following conclusions. The value of the sedimentation test in differential diagnosis between inflammatory and noninflammatory diseases and between

benign and malignant nevgrowths was confirmed but it must always be interpreted in connection with the other clinical findings. With this reservation the sedimentation test is valuable in urology in distinguishing between hypertrophy and cancer of the prostate and in the differential diagnosis of kidney colic. Litten's curve is recommended in the differentiation of the course of sedimentation. This curve shows that where there is greatly increased rapidity of sedimentation the severity of the pathologic process is shown in a particularly rapid fall in the terminal sedimentation. Sometimes in doubtful cases the sedimentation test can be judged only by the use of these curves. By repeated reading of the sedimentation time at certain intervals and by the course of the sedimentation a true picture of the course of the disease can be obtained and a more accurate prognosis can be reached than by other methods.

KIDNEY FUNCTION The Rate of Filtration and Reabsorption in the Human Kidneys
Rehberg P. B. Biochem Jour. London 1926, 19, 447

The filtration reabsorption theory is discussed. Its present state calls for a thorough modification, or for the complete abandonment of filtration as the main factor in excretion by the kidney.

The amount of creatinine present in the urine of man after ingestion of this substance is so large that it requires a filtration of up to 200 cc per min. to explain it.

The possibility of this is discussed and the result is taken to be in favor of the filtration theory.

The surface available for reabsorption in the proximal convoluted tubes alone is so large that provided it shows only the same power of reabsorption as the cloaca of the bird it may reabsorb the whole quantity of fluid required in the concentration process in the tubules.

The different ways in which a diuresis may be obtained according to the filtration reabsorption theory are enumerated and discussed with examples.

BISMUTH EFFECT ON KIDNEY A Study of the Blood Chemistry and the Histopathology of the Kidneys After Experimental Bismuth Injections. Brown H. Salesby E. R. and Schamberg J. F. Jour. Pharmacol. and Exper. Therap., August, 1926, xxviii, 165

These investigations were prompted by the increasing use of bismuth therapy in syphilis. A single product—potassium tartrobismuthate in olive oil—was used for experiments on rabbits from which it was found that

The histopathologic changes in the kidney sections correspond in general with the blood urea findings. The changes in one rabbit were severe, in another less severe, and in another the least changes were noted. It would appear, therefore, that blood chemistry tests would prove of value in controlling bismuth therapy in human beings.

These studies indicate that bismuth is a drug of relatively low toxicity. When one considers that as high as 30 mg. per kilo (9 times the therapeutic dose for the human species) were injected into rabbits without producing an alteration in the blood chemistry indicative of nephritis or histologic evidence of material kidney damage, the latitude between the therapeutic and toxic dose may be appreciated.

RETICULOCYTES The Clinical Significance of the Reticulated Red Cells. Damashek W. Boston Med. and Surg. Jour. April 29, 1926, cxviii, 759

Polychromatophilin, a universally conceded sign of regeneration in the fixed preparation runs parallel at times to the reticulation of the unfixed smear.

Apaetic anemia, which is an extremely rare condition, represents a rapid failure in bone marrow growth with resultant anemia and death. Accordingly the reticulate count would be expected to be nil at all and to remain so until death. The few cases that were observed bore out this assumption.

In purpura hemorrhagica in which there is a failure in platelet growth resulting in hemorrhages and anemia it was observed that when the reticulate count was low and remained so the patients continued to bleed and finally died. On the other hand when the reticulate count became high the platelets increased and the patient recovered.

Marked reticulosis in congenital hemolytic anemia is a pathognomonic point in the field of diagnosis, differentiating that disease from all other anemias with large spleens

In the anemias of pregnancy, the reticulate count acts as it does in the other anemias. In the leucemias and diseases of lymphoid origin, the reticulate count may be slightly elevated, but without relation to the course or scope of the disease

APLASTIC ANEMIA Differentiation of Aplastic Anemia and Essential Thrombocytopenia, McElroy, J B South Med Jour, May, 1926, 317, 325

Aplastic anemia is a severe myelopathy with deficient regeneration of the blood elements, characterized in many cases by extreme pallor of the skin, hemorrhages from the mucous membranes, petechiae and ecchymoses, absence of splenic enlargement, diminished hemoglobin metabolism, absence of plastic changes in the red blood cells, leucopenia and relative lymphocytosis, pronounced thrombocytopenia with lack of reaction from adrenalin and splenectomy, Rumpel Leed s phenomenon, prolonged bleeding time, nonretractility of clot, reduced bilirubinemia, and aplastic bone marrow, which resists all forms of treatment and in which splenectomy is positively contraindicated

Essential thrombocytopenia is a thrombolytic purpura, differing from the above in periodical occurrences of hemorrhages from the mucous membranes, frequently enlarged spleen, normal hemoglobin metabolism, presence of plastic changes in the red blood cells, increase of blood platelets after adrenalin and splenectomy, and a variegated bone marrow which is often benefited and frequently cured by splenectomy

	APLASTIC ANEMIA	ESSENTIAL THROMBOCYTOPENIA
I General Symptoms		
a color of skin	alabaster	variable
b skin and mucous membrane hemorrhages	usually pronounced	periodical occurrence with free intervals
c mouth cavity	frequently gingivitis, stomatitis, angina necrotans	numerous small hemorrhages, almost always gingivitis
d spleen	often small, not palpable	enlargement may be present
e urine	no urobilinuria	often urobilinogen
f gastric secretion	often disturbed	usually normal
g stool	very slight urobilin content	normal urobilin content
II. Blood Findings		
1 Morphology		
a erythrocytes	oligocythemia, progressive, poikilocytosis, polychromasia, reticulated cells absent	number variable, often evidence of regeneration
b leucocytes	leucopenia, marked reduction of granulocytes, relative lymphocytosis	often leucocytosis, differential count shows nothing distinctive
c blood plates	pronounced thrombopenia, (adrenalin and splenectomy do not produce an increase in thrombocytes)	during attack marked thrombopenia, after adrenalin and splenectomy an increase of platelets
2 Stasis experiment	skin hemorrhages	skin hemorrhages
3 Bleeding time	markedly prolonged	prolonged during attack
4 Retractility of clot	absent	absent
5 Color index	usually one	below one
6 Bilirubin content	reduced	normal

LABORATORY TECHNIC

BILIRUBINEMIA IN PREGNANCY The Diagnostic Value of Bilirubinemia in Pregnancy Mandelstamm A and Nagelkoff L. Monatschr f Geburtsh u Gynak, Jnné, 1926, lxxii, 297

The plasma is separated from citrated blood and 0.5 cc placed in a watch glass. One cc of 20 per cent trichloroacetic acid is added the mixture stirred with a glass rod and filtered through a small filter.

When the filter paper dries at room temperature, a positive reaction is indicated by a green color. With a negative reaction the sediment remains yellow or dirty white.

The reaction is always negative in normal cases. When used for the diagnosis of pregnancy, all factors leading to increased bilirubin in the blood, such as heart and liver disease, must be excluded.

The authors tested 134 cases and concluded that the test was a frequent but not constant sign of pregnancy. It is of no value in the differentiation of tubal pregnancy and adnexal inflammation as it may be positive in the latter.

HEMOLYSIN FORMATION The Formation of Antisheep Hemolytic Amboceptor in the Normal and Tuberculous Guinea Pig. Louis P. A. and Loomis D. Jour Exper Med October, 1926 xl 503

The guinea pig infected with virulent tubercle bacilli develops much more antisheep amboceptor than do controls when given like amounts of sheep red blood corpuscles.

The curve of antibody production in the guinea pig when treated with sheep red blood corpuscles shows a departure from curves previously determined in other animals.

These facts were ascertained as part of an effort to learn more of the functional nature of the inheritable factors controlling natural resistance to disease.

BLOOD SUGAR A Study of the New Benedict Method for the Determination of Blood Sugar. Rockwood R. Jour Biol Chem July 1926 lxxiv 187

The new Benedict method for blood sugar was compared to the Folin Wu in a series of 300 samples of blood.

The Benedict reagents which have been used up to the present time do not require so great a correction for lack of proportion in color development as the Folin Wu.

The normal cases in this group show a mode between 75 and 85 mg per 100 cc, and a range of from 50 to 120 mg.

The difference value between the Folin Wu and Benedict methods does not seem to be correlated with any factor in the condition of the diabetic patients.

The cuprous oxide precipitate in the Benedict test is less soluble than in the Folin Wu especially with high blood sugars.

The substance causing the difference value is apparently not one of the ordinary nitrogenous reducing substances since the difference value is not increased in uremia.

Sugar tolerance tests show no characteristic variation in the difference value.

The substance causing the difference value seems to be present in greater quantity in the cells than in the plasma and is sometimes only present there.

SPIROCHETA PALLIDA Congo Red and Hydrochloric Acid or Nitric Acid (Faming) Method for Treponema Pallidum Examination, Udasco L. Jour Philippine Med Assn, June 1926 vi 196

"(a) Take a small drop of secretion from a suspected lesion and deposit it on one end of a clean slide. Emulsify it with an equal quantity of Congo Red (2.5 per cent in water). Make a smear as for blood. Air dry.

"(b) Pour enough concentrated HCl or HNO₃ in a small Petri dish, or similar container and place the slide on the rim of the dish smeared surface down so as to come

in contact only with the fumes of the acid. Almost instantly the smear turns blue and in thirty seconds is ready to be examined under oil immersion objective. The *Treponema pallidum* will be found as clear white spirals against a uniformly blue background."

GLUCOSE ESTIMATION On the Estimation of Glucose in the Presence of Phosphate Buffers, Visseher, M. B. *Jour Biol Chem*, July, 1926, lxx, 9

It is pointed out that the presence of potassium acid phosphate in glucose solutions lowers the reducing power of the glucose for copper. This fact must be taken into account whenever the sugar content of buffered solutions is measured.

SYPHILIS, FLOCCULATION TEST WITH DRIED FISH EXTRACT Chemical Application of Flocculation Reaction With Katsuobushi (Dried Japanese Fish) With Blood Serum and Plasma in Syphilitic Children, Takeda, S. *Oriental Jour Dis Infants*, July, 1926, i, 81

The author cut up Katsuobushi, mixed it with ether, and let it remain for three days at room temperature (15° C), changing the ether several times. After removing the ether, he added 85 per cent alcohol in proportion of 1:5, after which the mixture was allowed to stand for one week at room temperature and then filtered.

The test gives results approximating those of the Wassermann in 85 per cent of 160 cases. This test was also tried in 400 cases by Sasaki (Sasaki, R. *Oriental Jour Dis Infants*, July, 1926, i, 82) with an agreement of 89 to 90 per cent in activated or inactivated sera and an agreement with Wassermann reaction of 88.75 per cent.

DIPHTHERIA BACILLUS The *Corynebacteria* (Diphtheria Bacillus and "Diphtheroids") The Importance of Microphotography as an Aid to Their Classification and Identification, Thomson, D., and Thomson, R. *Annals Pickett Thomson Res Lab*, London, 1926, ii, 51

The authors comment upon the fact that there are hundreds rather than a score of "diphtheroids" whose satisfactory classification is a matter of great confusion and difficulty, which has been farther intensified by the habit of applying definite names to diphtheroids which have been inadequately described.

They contend that the clearest, most easily and rapidly comprehended, as well as the best method of recording the diphtheroids in an orderly and accurate manner is to give a careful and complete list of tests and characteristics of each, accompanied by as many good photographs as possible.

In pursuance of this plan, in a paper covering 149 pages, they present such a study of the diphtheria and "diphtheroid" strains (72) embodying complete cultural and microphotographic characteristics.

The paper is illustrated with 130 microphotographs which well repay study.

Their study involves routine culture on the testicular infusion peptone glucose agar (*Annals Pickett Thomson Res Lab*, i, 223) and on the following sugars: glucose, maltose, galactose, saccharose, lactose, mannite, and dextrin. Frequent microphotographs of both Gram stained specimens and growth in culture form the remainder of their basis for classification.

The paper contains a wealth of data and can be studied with profit.

PRECIPITATION TEST FOR SYPHILIS A Simple Rapid Precipitation Test for the Diagnosis of Syphilis, Butler, H. W. *New Orleans Med and Surg Jour*, August, 1926, lxxix, 105

Antigen Preparation—Fresh baby veal hearts are selected, and the superficial fat is removed. The muscle is ground in a sausage grinder, spread on paper and dried with the aid of an electric fan. After it is completely dried, it is powdered in a mortar and extracted with ether. Four hundred cc of ether are used to each 100 gm of powdered heart. This is allowed to act for ten minutes, shaking frequently, and the ether is then

filtered off. The material is extracted again three different times with 300 cc of ether which is filtered off and discarded in each instance. The ether extracted powdered heart muscle is now dried free from ether, and for each gram of the dry material 5 cc of 95 per cent alcohol are added and maceration is allowed to continue for three days at room temperature after which the alcohol is filtered off and enough 95 per cent alcohol added to bring it up to the original volume. This constitutes the defatted alcoholic heart extract for the antigen. Six decigrams of cholesterol and 3 cc of glacial acetic acid are added for each 100 cc of the alcoholic extract. This is filtered after solution is effected and constitutes the finished special antigen for this test. This antigen seems to be stable for at least several weeks.

Technic—One cc of antigen is measured into a test tube and 2 cc of distilled water into a second tube (normal saline can be used but the solution is unstable). Mixing is effected by pouring the solution from one tube to the other back and forth for at least six times.

Two drops of serum are placed upon a clean slide about the junction of the middle and outer third. With a pipette used only for the antigen dilution three drops of the dilution are placed upon the slide near but not into the serum. These are mixed on the slide thoroughly with the end of a toothpick or other suitable instrument and the slide is slowly rocked for two minutes. If the serum is positive a characteristic granular precipitate which can be easily seen develops during the rocking process. If negative no specific precipitate forms within the two minute time limit.

SPIROCHETA PALLIDA, STAINING OF Staining Spirocheta Pallida in Sections by Silver Impregnation. Lukes J. and Jelinek V. *Ztschr f Immunol. u Exper Therap*, April 8 1926 lxiv 83

The authors have had good results with the following method:

Formol fixation. Alcohol twenty four hours. Place in water until the pieces fall to the bottom. Silver nitrate 2 per cent for two or three days. Short irrigation with distilled water. Thirty per cent grape sugar solution for two days. Place in water for 'a long time'. Frozen sections or embedding.

They also used with success the solution below instead of glucose as an oxidizing agent:

Onion skins are macerated and boiled for a long time then they are pressed and filtered several times until the fluid becomes clear. This is allowed to ferment and is then sterilized and filtered.

If a rapid method is desired sections that have been well dehydrated in alcohol (up to two hours) are put in distilled water and left until they fall to the bottom. They are then put in 2 or 3 per cent silver nitrate for three hours at 56 and then washed in water and reduced in onion stain for two or three hours at the same temperature. The sections are then washed with alcohol balsam.

ANEROBES A New Method for the Cultivation of Anaerobic Bacteria With Cystein in Media, Hosoya, S. *Japan Med World* April 15 1926 vi 83

Broth containing 0.001 per cent of L-cystein hydrochloride adjusted to P_H 7.2 to 7.1 gives a profuse and rapid growth of obligate anaerobes without a vaseline seal.

OVA IN FECES A Method for the Routine Examination of Feces for the Ova of Parasitic Worms and Encysted Amebas, Johns, F. M. *New Orleans Med and Surg Jour.*, 1926, lxxix, 218

Formed or semisolid stools are diluted from 10 to 15 times with water shaken well, and strained through two layers of cheesecloth directly into a 12 by 115 mm. ($\frac{1}{2}$ by 4 inch) round bottom tube. Balance tube in centrifuge (This should be equipped with Cornell shields and should be able to run at least 1500 r.p.m.) Start centrifuge and immediately advance to full speed of 1500 r.p.m. Shut off power within from fifteen to twenty seconds after

advancing the speed Pour off the supernatant fluid, resuspend sediment with water and re-centrifuge (Another such washing will frequently clear the sediment still more) Decant again, leaving a few drops of water with which to shake up the sediment Pour on slide and spread out to where all particles on the slide are easily visible under the low dry lens and examine as for parasite ova

DUCREY BACILLUS Technic of the Isolation of the Streptobacillus of Ducrey, Durand, P Compt rend Soc de biol, Paris, June 4, 1926, xciv, 1324

Media—1 Agar with sheep's blood One hundred and fifty cc of ordinary agar (3 per cent) in peptonized beef bouillon of about P_H 7.6 previously sterilized in an Erlenmeyer flask with a capacity of 250 cc is placed over a water bath until completely melted This nutritive medium is then cooled to about 45° C and from 30 to 50 cc of fresh defibrinated sheep's blood is added Sheep's blood which has been kept aseptically may be used The medium is divided into inclined tubes which may be used in a few hours or as long as they are not too dry

2 Pure sheep's blood Sheep's blood obtained aseptically from the juglar vein is immediately divided into tubes 11 cm by 11 mm which are placed in an inclined position to favor coagulation At the end of a few hours they are placed in an upright position, and the serum soon separates from the clot

These tubes may be used as soon as the serum exudes and separates from the clot, but it is better to wait for half a day They remain good as long as they have not dried out, even if the serum is tinted with hemoglobin Both media should be stoppered and kept in an ice box if they are not to be used for some time

If the bubo is not opened, puncture should be made with a large needle on a tight syringe If the bubo is open, paint it with tincture of iodine after slight pressure, then apply a tampon wet with alcohol The sample is taken from the interior with a pipette In the case of chancres, if possible, select one in the state of a pustule which has not yet opened Touch with tincture of iodine, then with alcohol Remove the epidermal layer aseptically and obtain the pus with a platinum wire or a pipette When the chancres are wide open, the whole surface must be sterilized and the streptobacillus obtained from the deeper layers In order to do this, clean carefully with gauze, then apply a tampon of tincture of iodine for three or four seconds, wash rapidly with alcohol, and dry with gauze The chancre is then squeezed with the fingers until a serous fluid exudes This fluid, which may be blood tinged, is picked up with a pipette

The agar with sheep's blood is an excellent medium for the bacillus of Ducrey, but it is also favorable for many saphrophytes, hence this medium is used for clean products (unopened buboes or chancres) Less material is required and more tubes are to be used, and the material is to be spread over the whole surface of the tube when the lesions are open

The tubes of pure blood are less favorable for an abundant development of the streptobacillus, but it is less favorable for other bacilli The material should be implanted in the serum, not in the clot

The tubes are placed in an incubator with a rather low temperature, preferably 30° to 36° C On blood agar typical colonies of the Ducrey bacillus develop in from thirty six to forty eight hours On pure sheep's blood the growth should be looked for in stained preparations The bacilli are very few in number, and they often show involution forms As soon as they appear, they should be transplanted on tubes of blood agar

Positive results were obtained in 39 out of 40 cases by these methods

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building
Richmond Va

New Books Offered to Profession

THE new Illustrated Catalogue just issued by W B Saunders Company medical publishers of Philadelphia and London, describes and illustrates more than 250 titles. Of these, many are important new books or new editions not described in the former issue of their Catalogue.

A serviceable feature of Saunders' Catalogue is the giving of the month and year of publication of each book listed. This together with the description and in many cases the table of contents, author and price makes the Saunders Catalogue one of unusual accuracy and precision from which the doctor may select and order his medical books.

A number of new books in this Catalogue deserve the particular attention of progressive physicians and surgeons. For instance there are Blumer's *Bedside Diagnosis*, McLester's *Nutrition and Diet*, Bransch's *Urography*, Cecil's new *Textbook of Medicine*, Stokes' *Clinical Syphilology*, Kolmer's *Chemotherapy*, Ford's *Bacteriology*, Young's *Urology*, Rehfuss' *Diseases of the Stomach*, Pelouze's *Gonorrhea in the Male*, Crohn's *Affections of the Stomach*, Ewing's *Neoplastic Diseases*, de Takats' *Local Anesthesia*, Wechsler's *Clinical Neurology*, Palfrey's *Specialties in General Medicine* etc etc. Anyone desiring a copy of this 80 page Catalogue needs but indicate his wish to Saunders Company and one will be sent him immediately. It is worth having in the library.

*Standard Methods of the New York State Board of Health**

IN THIS volume are presented in detail the methods used in the various departments of the New York State Board of Health.

This volume is divided into the sections listed below.

I *General Laboratory Procedures* in which the handling of specimens, the preparation and use of stains, and the handling of experimental and test animals are succinctly but clearly outlined.

II *The Preparation of Media and Glassware*—In this section the directions are precise and somewhat elementary, as they are intended for workers, as a rule unfamiliar with laboratory regulations. A variety of media are described and this section alone will be a useful source of reference in laboratories in general.

III *Methods Used in the General Diagnostic Laboratories*—This section while intended for those having some experience in laboratory technique, is nevertheless complete and not lacking in detail.

One regrets that in view of the purpose of the book Orskov's method of single cell culture is not described but merely referred to as reliable and simple nor are there detailed directions for micrometry or dark field examinations. One notes also that hemoglobin is reported in per cent, in spite of the inaccuracy this involves.

Standard Methods of the Division of Laboratories and Research of the New York Board of Health. By A. B. Wadsworth. M.D. Cloth. Pp 704. 56 Illustrations. 1 plates. Williams and Wilkins Co. Baltimore.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

IV *Methods Used in the Laboratories for Sanitary and Analytic Chemistry*, in which are described the examination of water, sewage, effluents, wastes, ice, milk, and cream

V *Methods Used in the Antitoxin, Serum and Vaccine Laboratories* In view of the empiricism of laboratory practice in this field, this section will be of great value and will be read with great interest by those engaged in this work In view, also, of the empiricism not uncommonly encountered in the use of such agents the section could perhaps also be read with profit by many practicing physicians This is the longest section of the book

The last two sections are concerned with the methods used in the executive, research, publication, and library departments

All in all the book is a valuable and noteworthy publication and typographically exemplifies the motto of the publisher

*The Tongue and Its Diseases**

AN ENGLISH surgeon covers the tongue and the diseases to which it is heir in textbook proportions There is probably no part of the body about which the average practitioner of medicine knows less Although written from the surgeon's viewpoint, the internist will find this volume a very useful addition to his reference library

"Perhaps the chief lesson of this book is that cancer of the tongue, in place of being the hopeless disease that the laity and many physicians and some surgeons seem to think it is, is just as curable in its early stages as is cancer elsewhere"

The volume contains a wealth of interesting, as well as useful, illustrations

The Ear, Nose, and Throat in General Practice†

AS IN other works of its kind, the author, a specialist, frequently overshoots the bounds of general practice It is in no sense a textbook, or one for reference After perusing its pages one feels as if he has been listening to fatherly advice There are excellent chapters on the general practitioner's opportunities and responsibilities in acute ear disease The nose is probably not so well done Foreign body in the food and air passages is touched on as well as the technique of tracheotomy and diagnosis of acute and chronic sinusitis Tonsils and adenoids are not overlooked, in fact the former is overdone from the practitioner's viewpoint Space is judiciously apportioned to the more uncommon conditions

The author's plea for early diagnosis and prompt action in acute ear disease is earnest and convincing

The fact that many common diseases of the ear, nose, and throat are preventable places a great responsibility on the family doctor In the direction of the life of a patient, the specialist is practically helpless It is the general practitioner who must impress upon his patient the blessed value of "facing up to" the weather, apply silver nitrate in his first attack of tonsillitis, or apprehensively see him with his first earache

The Diagnosis of Pancreatic Disease‡

A BRITISH chemico-clinician's views on the manifestations of pancreatic pathology rather tersely and intelligibly stated The work presents an anatomic, physiologic, and pathologic background, which although not treated in an exhaustive manner, furnishes satisfactory

**The Tongue and Its Diseases* By Duncan C L Fitzwilliams CMG, MD ChM FRC S Edin and Eng Surgeon and Lecturer on Surgery to St Mary's Hospital Paddington Green Children's Hospital and Mount Vernon Hospital Cloth Illustrated Pp 505 Oxford University Press American Branch New York 1927

†*The Ear Nose and Throat in General Practice. An Informal Guide to the Main Principles* By D A Crow MB ChB (Edin) Oto-Laryngologist The Royal Sussex County Hospital Brighton The Sussex Throat and Ear Hospital Etc Illustrated Cloth Pp 150 Oxford University Press American Branch New York 1927

‡*The Diagnosis of Pancreatic Disease* By Robert Coope MD BSc MRCP Senior Assistant Physician Royal Southern Hospital Liverpool Assistant Physician Hospital for Consumption and Disease of the Chest Liverpool Lecturer in Clinical Chemistry University of Liverpool Cloth Pp 112 Oxford University Press American Branch New York

orientation. Of course a classification of pancreatic lesions is included. Acute pancreatitis is covered expeditiously in order that the main theme chronic pancreatitis may be arrived at logically. The latter is rather comprehensively treated under the following headings: clinical observations including x-ray findings; attempts to detect failure of the discharge of pancreatic juice into the duodenum; attempts to detect failure of insulin production; the findings and opinion of a surgeon during an abdominal operation; the therapeutic test; and esoteric tests with dubious foundations. About one hundred and seventy references are included.

The author is somewhat dogmatic at times, criticizing and denouncing diagnostic methods which he admits never having tried. He believes the most important test of chronic pancreatic dysfunction to be the finding of an abundance of striated muscle fibers in the feces of persons receiving an optimum of meat in their diets. This is very encouraging for the busy internist or surgeon who becomes lost in a maze of complicating techniques.

On the whole it is a very delightful essay on the Cudgeling of the Abdomen.

The Internal Secretions of the Sex Glands

NOT a series of popular exaggerations and unwarranted theories but a critical review of the scientific literature on the subject into which is incorporated the author's own experiments, experiences and interpretations. His references to the literature are most extensive and include work done and observations made not only on man but on a wide variety of animals such as dogs, fowl, fish, amphibians and insects. The work might be characterized as a study of the comparative physiology and pathology of the internal secretions of the sex glands. It will have its largest audience among the endocrinologists.

The author presents a summary of the literature dealing with the chemical aspects of internal secretions. Having an interest in the subject, the authors were impressed by the absence of any collective account of the original contributions on the subject and thus they present in the work under review. In it will be found a description of the history, methods of preparation and chemical and physiological properties of the various internal secretions.

The Transfusion of Blood†

IT HAS been said that the first transfusion was performed in 1492 by a Jewish physician who gave blood to Pope Innocent VIII. According to the story the blood was obtained by bleeding three boys to death. This is probably fiction since knowledge that the blood circulates dates only from 1668. In the Middle Ages the value of blood as a health restorative was recognized but during these times it was taken by mouth. The first successful transfusion of blood in animals was performed in 1665; the first in man was in 1667. In this case the blood of a lamb was injected into the veins of a boy who curiously enough recovered. Animals were often used thereafter with many consequent deaths so that after a time blood transfusion was forbidden in France. Even after a reasonably satisfactory method of blood transfer from man to man had been developed (1835) many deaths resulted and it was not until 1907, when we learned that there are at least four groups or types of blood which can be readily identified and some of which are incompatible with each other that transfusion was placed on a firm basis. Since then preliminary blood matching has practically obliterated serious transfusion reactions. The more extensive utilization of this therapeutic measure has since depended upon improvements in operative methods. The earlier arrangements were crude indeed when compared with the present day technique and attempts

The Internal Secretions of the Sex Glands. The Problem of the Puberty Gland. By Alexander Lipschutz, M.D., Professor of Physiology in Dorpat University (Formerly formerly Privat-docent of Physiology in Bern University (Switzerland)). With a Preface by F. H. A. Marshall, F.R.S., Author of *The Physiology of Reproduction*. With over 140 illustrations in the text. Cloth, pp. 513. William & Wilkins Co., Baltimore, Md.

†*Transfusion of Blood.* By Henry M. Feinblatt. Cloth, pp. 156. MacMillan Co.

at transfusion even in expert hands were often unsuccessful. This was chiefly due to the tendency of the blood to clot before completion of the transfusion.

In 1914 anticoagulants were first satisfactorily used in clinical work so that blood could be drawn into receptacles where it would not clot. With this method the donor and the recipient need not be in the same room, nor indeed in the same building. The vast number of these so called indirect transfusions during the World War indicated convincingly the great value of blood administration in certain conditions, and this experience went far in popularizing the procedure.

However, the generally accepted opinion has been that whole blood is more effective than blood which has been treated to prevent coagulation, and within the last few years instruments have been devised which have so simplified the direct method that it has come into quite general use in those cases in which transfusion is indicated. The present day procedure possesses the additional advantage that it is not necessary to cut down upon veins and arteries thereby destroying them for future use. So satisfactory are these methods that one group of authors was able to report in 1926 the carrying out of 1000 transfusions all of which were successful as far as the transfusion was concerned.

Blood transfusion is often indicated in a wide variety of conditions, such as sudden loss of blood from any cause, surgical shock, illuminating gas poisoning, chronic hemorrhagic diseases, as a preoperative precaution when the bleeding and clotting time of the patient's blood has been found to be delayed, in bacterial infection, debilitating conditions, diseases associated with blood destruction, and the like.

Dr Feinblatt contributes a valuable monograph on blood transfusion in which after an historical review and a study of the various methods, he describes in detail his own method, which may certainly be recommended for simplicity and efficiency.

*Technic in the Management of Diabetic Patients**

ACUPUNCTURE has become such a prominent part of the medicine of today and the home administration of hyperdermics has become so widespread since the advent of insulin that haphazard technic, unnecessary jabbing, and the use of crude instruments, such as improperly sharpened needles, is readily detected by the observant patient.

Skill in this line is chiefly a matter of experience and repetition, but there are many little tricks which will promote success and which the author describes in his small brochure. No technician or physician will read it without getting at least one or two little points which he had not previously realized. The author also incorporates chapters on the preparation and intravenous administration of glucose, transportation of blood, and a practical outfit for urinalysis.

Gastric Function in Health and Disease†

RYLE believes that while hunger is associated with peristaltic contraction of the stomach wall, appetite depends upon the degree of its muscular tone. It is the flaccid, atonic stomach that is associated with anorexia, while the tonic or hypertonic organ is usually accompanied by good or abnormal appetite. Appetite is a function of muscular tonicity. Hunger is a function of muscular contraction.

An analogy exists in the skeletal muscles. Good muscular tone gives its possessor a sensation of well being and an inclination to active movement. Actual contraction of the muscles results in the movement.

Recent work would indicate that the level of gastric acidity is not maintained so much by the amount of secretion of hydrochloric acid as it is by the extent of alkaline regurgita-

*Technic in the Management of Diabetic Patients. By Henry J. John. M.A. M.D. Cloth. Illustrated. Pp. 62. The Williams Feather Company. Cleveland, Ohio. 1927.

†Gastric Function in Health and Disease. By John A. Ryle. M.D. (London). F.R.C.P. Assistant Physician and Lecturer on Medical Pathology. Guy's Hospital. Cloth. Pp. 152. Humphrey Milford—Oxford University Press.

tion from the duodenum. Thus, in gall bladder disease there may be enacidity or even anacidity, in the past erroneously interpreted as a true achylia gastrica. But this may be due entirely to excessive duodenal regurgitation. It is pointed out that without chloride estimations, it is impossible properly to interpret hyperchlorhydric and achlorhydric reactions. When free acid is in excess, chloride estimations alone can distinguish between a positive hypersecretion and a failure in neutralization. The important conditions promoting pathologic hyperacidity are (a) excess of secretion, (b) pyloric hypertonus preventing duodenal reflux, (c) too rapid emptying. The secretion of even a normal gastric juice into a too rapidly diminishing volume would necessarily entail a steady rise in the percentage of acidity.

Ryle believes that acid in the duodenum is not an important factor in citing closure of the pylorus as has been taught. Others have shown with simultaneous gastric and duodenal intubation that acid in the duodenum does not necessarily close the pylorus. The most rapid emptying took place at a time when the duodenal contents were unusually acid. Carlson has found that not only acid but mechanical and even alkaline stimulation of the duodenum can produce pyloric closure. Possibly gastric emptying is entirely similar to peristalsis elsewhere, stimulation at one point producing contraction or increased tension above, with relaxation below. A tonic phase in the pylorus is followed by a phase of relaxation which, especially in the later stages of a meal, allows fluid regurgitation when the next lower portion, the duodenum, becomes tonic.

The pain of gastric ulcer is evidently not due to hyperacidity. It appears to be an expression of hypertonus, excessive peristalsis and inhibition of pyloric relaxation, all of these factors working together to increase intragastric tension. Even the feeding of hydrochloric acid in ulcer will not increase the pain, and it may persist with complete anacidity.

Hurst remarks that hunger pains develop when only a small portion of the food is still present in the stomach and the hypertonic condition, constantly present in cases of duodenal ulcer, reaches its greatest development.

Pain disappears when tonus relaxes after the introduction of food, fluid or gas into the stomach. Ryle suggests that the beneficial effect of sodium bicarbonates may be due chiefly to the rapid evolution of gas with consequent effective relaxation of tonus. Effervescent alkalis give more relief than do noneffervescent alkalis. In the absence of free acid, alkalis fail to give relief in ulcer, not because the pylorus is incapable of relaxation but because in the absence of acid no gas is liberated.

This theory of the cause of delayed pain in gastric or duodenal ulcer will explain the development of ulcer symptoms in nervous dyspepsia. Worry, overwork, the constant go of the business man, may produce symptoms indistinguishable from those of ulcer which, however, clear up entirely with adequate rest or following a vacation. Here the primary factor was increased tonus. Ryle believes that sodium bicarbonate relieves the immediate pain of lesser curvular ulcer mainly by facilitating belching and thereby lessening tension in the proximal portion. Ryle discusses the etiology of symptoms associated with gastric dysfunction such as flatulence, nausea, belching, etc. His discussion of the results of gastroenterostomy and the causes for persistent symptoms will be of interest to all clinicians.

Food Values*

THIS is a quick reference manual for nephritics and particularly diabetics who are on weighed and calculated diets. For each article the compiler has indicated the food value for different quantities. One may find at a glance the quantity of protein, fat, and carbohydrate and the caloric value of five grams, ten grams, twenty grams, thirty grams and so on up to one hundred grams. This greatly facilitates the preparation of weighed diets. All of the foods commonly used are provided for in this book.

In addition, wherever practicable the author has indicated under each food the weight in grams of the usual portion of the food as customarily served.

Food Values—For Calculating Diabetic and Nephritic Diets. Calculated from Bulletin No. 8 U. S. A. Department of Agriculture. By Louise M. Keegan, University of Chicago Dietitian for Polyclinic Hospital, New York City. Cloth. Pp. 106. The Macmillan Company.

In an appendix we find the usual supplementary information with regard to methods of weighing food, conversion tables, simple urinalyses, and the preparation and administration of insulin

This book should find its greatest usefulness in the hands of the diabetic patient and the hospital dietitian

A Guide for Diabetics†

LIKE all diabetic manuals this contains the usual necessary information for patients, such as description of the disease, food principles, measurement of food, principles of dietary treatment, use of insulin and its dangers, general hygienic treatment, urinalysis, food analyses, etc

In two outstanding features it differs from the other manuals. The last chapter is devoted to home canning of food supplies. This is reminiscent of the helpful work of Sprigge and his coworkers in Duff House Papers (Oxford Press) in which home gardening for diabetics is described in detail.

But the great interest of the book of Campbell and Porter lies in its simplification of the dietary calculations to be made by the patient. After the physician has drawn up a permanent basic sample diet appropriate to his patient's needs, containing eggs, bacon, roast beef, boiled ham, cheese, and custard, the patient may then substitute an almost endless variety of foods for any amount of one or more of these basic ingredients by simply referring to a most exhaustive *table of equivalents*. No calculation is necessary after the calculation of the standard basic diet.

Defective Memory—Absentmindedness and Their Treatment‡

THE first portion of this book consists in a popular presentation of the psychology of memory. The information is consistent with our psychological knowledge.

"If I desire to recall something to mind, I must first fix it there. A thing which has not been fixed cannot be remembered. If, however, I desire to remember a thing well, I must focus on it. At this point will power becomes necessary. A man who lacks this is unable to concern himself long enough with an object to concentrate his entire attention upon it and to observe it from every angle. His observation of it will be fleeting, his fixation of it but superficial, and consequently his remembrance of it will be but short."

"Easy distraction is a great detriment to the capacity for attentive fixation."
 "Memory in the case of the aged cannot usually be perfect because the sense organs are dulled and no true images can be formed."

"If a person or thing is looked at carefully, examined critically from every side, and so well fixed in the mind because a perfect image of it is formed, there will be no trouble in keeping it in the memory."

Memory is of two kinds, repetitive and associative. The former is based upon constant repetition of the idea to be recalled and the latter upon its association with other ideas, surroundings, or similar situations. The best memory consists of that developed by a combination of the two.

The author establishes his idea that in most instances memory impairment is but a symptom and is associated with functional or organic disease, such as cerebral arteriosclerosis, intestinal toxemia, thyroid deficiency, deficient secretion of the other endocrine glands, constipation, infection, pathology in the nose and the like. The greater part of the volume is therefore devoted to a discussion of the treatment of these medical complications.

**A Guide for Diabetics*. By Walter R. Campbell. University of Toronto and Mame T. Porter. Toronto General Hospital. Cloth. Pp. 259. The Williams and Wilkins Company. Baltimore, Md. 1926.

‡*Defective Memory—Absentmindedness and Their Treatment*. By Arnold Lorand. M.D. Carlsbad, Czechoslovakia. Author of *Old Age Deferred*. Cloth. Pp. 340. F. A. Davis Company. Philadelphia.

A supposed relationship between diseases and the type of forgetfulness is brought out. Thus, the author states that in senile dementia disturbances in the power of fixation are more pronounced, while in general paresis it is the disturbance in the power of recollection that gives the trouble. Again in neurasthenia the power of fixation is frequently affected in the highest degree entirely as a result of the ennui and listlessness of the neurasthenic who takes little or no interest in things about him.

In the treatment of the systemic diseases mentioned the author follows almost without exception the generally accepted therapeutic measures. We are glad to note that Dr. Lorand's attitude on gland therapy and particularly the more recently recommended operations for rejuvenation is thoroughly conservative.

The treatment of defective memory consists in improving the patient's general physical condition. In addition to this in certain types of cases good results are claimed for thyroid therapy, ovarian therapy, by treatment of cerebral arteriosclerosis and the like. The author outlines his own plans for training the memory. As usual these are based principally upon fixation of the attention and upon the calling up of associative memory pictures.

One of the enjoyable features of the volume is its wealth of anecdote on the memory of important personages in history.

*The Conquest of Disease**

WHEN we sigh for the good old days when men were men, all women were fair, and life was aglamour with romance, we shall do well to think of the toothless hags of forty, the decrepit rouns of fifty, the open sewers running down the center of all paved streets, the filth, the ignorance and general suffering in all but modern times.

The influenza epidemic of 1918 occurring within our own times was to us the greatest plague which the earth has ever borne. But let us turn back the pages of history. The plague in 80 A.D. is said to have destroyed as many as 10,000 in a single day in Rome which was then a city of about 1,000,000 inhabitants. The 'black death' of the fourteenth century is still without parallel. In it Cairo lost daily from 10,000 to 15,000 souls. In China more than 13,000,000 are said to have died of it. Cypress lost all of its inhabitants and ships without crews were often seen in the Mediterranean. In Avignon the Pope found it necessary to consecrate the Rhone that bodies might be thrown in without delay as the churchyards could no longer hold them.

The greatest castles, built by feudal lords had no such convenience as a bathroom or any proper means of disposing of human filth. Excrement was thrown into the surrounding moat and it is barely possible says Rice that the stench arising from the moat was of value in keeping away enemies. No wonder says Rice that heavy perfumes and smelling salts were much in demand. As a special privilege nuns of certain orders were permitted to bathe once a year, but the immodest practice was not encouraged.

Nineteen hundred and twenty-seven is a happy time in which to live. But how did it become such? Chiefly through great medical and sanitary discoveries and their practical application in public health and preventive medicine. Dr. Rice has written a most readable popular exposition on preventive medicine. It may be read with pleasure by the physician and with the greatest profit by the intelligent layman. The illustrations are plentiful, well executed and informative. There comes first a description of the scientific conquests of transmissible disease, the cause of transmissible disease, infection and resistance, then a detailed description of those various diseases which are usually studied and discussed by epidemiologists. The last section deals with the means by which transmissible diseases are controlled, such as vital statistics, segregation, isolation and quarantine, disinfection, sanitation and public health administration.

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EDITORIALS

Chronic Appendicitis

THE early surgical knowledge of disease of the appendix consisted in recognition of the fact that some people with severe, acute pain in the right lower quadrant of the abdomen later developed abscesses in this region which could be drained surgically. With the advent of abdominal surgery the idea gradually gained acceptance that the acutely inflamed appendix was an early stage in the development of abscess and that its removal would prevent this sequel. It soon became apparent that not all cases of acute appendicitis went on to abscess formation, and the conclusion gained acceptance that the disease may be intermittent, with irregular flare-ups and intervening periods of quiescence or relative quiescence.

The fourth conception to develop was that there need not necessarily be an antecedent acute attack but that the organ may from the first be the site of low-grade chronic inflammatory processes responsible for both local and remote symptoms and organic alterations. Many and diverse ills have been attributed to chronic appendicitis.

Unfortunately far too many patients operated upon in the name of chronic appendicitis have experienced no relief from their symptoms, even though the appendix has been removed. The physician at first saw many more of these unsuccessful results than did the surgeon, but as a large percentage of the patients later required or had operations for other pathology apparently causing the same symptoms, the surgeons also gradually became aware of the prognostic disadvantages of operating merely for so called chronic appendicitis. This led many of the more inquisitive among them to carry out extensive follow up work on their own patients from which they were able to draw conclusions as to the ultimate benefit to be derived from operation.

So much is known of the pathology and treatment of appendicitis in general that when we see reports of symposiums on appendicitis, we conclude at once that they were presented before some county medical societies. That all is not well in our understanding of chronic appendicitis is now indicated by a symposium on this subject presented before the section of surgery at the 1927 meeting of the British Medical Association.

Both sides of the argument were well presented. Wilfred Trotter admits the existence of chronic appendicitis without preexistent acute attacks. Such an appendix may be abnormally long, is usually abnormally fixed either by its mesentery in its proximal half or by being tied down to the posterior wall of the cecum, its caliber is not uniform but shows some dilatation usually toward the free end, and it contains elongated, separate more or less firm fecal masses, while its mucous membrane looks swollen and spongy. The lymph glands at the root of the appendix and in the ileocecal angle have the subglobular shape and glassy look of chronic inflammatory enlargement. The cecum is usually large, inert, and contains solid fecal material. Trotter stresses the evidence of septic absorption shown by enlarged lymphatic glands and the evidence of disturbed motor and perhaps secretory functions shown by the inability of the appendix to empty itself. Glandular enlargement is common at all ages, but especially in young adults. He suggests that it is probably as common in appendicitis as in tonsillitis but that it is often not looked for. He believes that acute lymphadenitis is a frequent event in the chronic appendicitis of children and an important cause of local symptoms. Gastric symptoms, flatulence, constipation, and sometimes diarrhea are attributed to disturbed peristaltic functions resulting in nerve irritation in the appendix.

Victor Bonney attributes the frequent operations for chronic appendicitis in women in part at least to faulty diagnosis. Three gynecologic conditions in particular are responsible for this: first, disease of the right fallopian tube, second, cyst of the right ovary particularly blood cysts (the so called chocolate cyst), and third, retroversion of the uterus with its resultant drag on the ligaments about the cecum, always more definite than that on the looser attachment of the colon in the left side.

A. J. Walton believes that primary chronic appendicitis is a doubtful entity. He, like Willys Andrews, believes that a chronic appendicitis should never be diagnosed unless there has been at least one acute attack. The chief pathologic changes found in the so called chronic appendix are a diffuse fibro-

sis of the appendix with obliteration of the lumen, usually commencing at the tip and steadily progressing downward, and the presence of periappendicular bands, binding the appendix to the cecum and under surface of the mesentery. He believes that these are degenerative or involutionary rather than inflammatory changes. He attributes the greater frequency of pain in the right lower quadrant in women in part to greater frequency of visceroptosis and mobile cecum. Shellen has stated that pain commencing in the right iliac fossa is not due to chronic appendicitis. Maclaren has observed that the appendix is frequently removed for chronic appendicitis with unsatisfactory results, but he distinguishes the so-called chronic appendicitis from the relapsing type. Stanton investigated a series of 100 patients who had been operated upon for chronic appendicitis and found that only 65 patients had been relieved of their symptoms.

In Walton's experience all those cases of so-called chronic appendicitis with pus in the lumen of the appendix, dense adhesions or strictures with concretion formation had had at least one previous acute attack.

In this country Robert Morris has offered a classification of five kinds of chronic appendicitis. He states that the most frequent kind is that which leads to the largest number of mistakes in prognosis and which furnishes a great group of worthless appendix operations. It is an irritative lesion belonging to the normal involution of the appendix, consisting essentially of connective-tissue fibrosis. He believes that this involutionary process occurring in the appendix in young people is a stigma of physical decline and is but a part of a group of stigmata which may be readily discovered. He speaks of the short sternum with ptosis of abdominal viscera, loose kidney, narrow costal angle, crowded teeth, and the like. Removal of the appendix does little good in this type of condition, which in general he classes as neurotic.

The other types of chronic appendicitis in his classification are, appendices showing scar tissue following an antecedent acute appendicitis, chronic infectious processes involving the cecum as well as the appendix and sometimes associated with entozoa in the appendix, lymphoid hyperplasia of the organ, and chronic congestion associated with chronic congestion of other parts of the bowel related largely to blood or lymph circulatory disturbances.

Hertzler has recently presented a pathologist's viewpoint. He is most emphatic in his conclusion that the so-called primary chronic appendix does not exist. Such changes as exfoliation of the epithelium, hemorrhages in the lumen, diminution in the number of goblet cells, moderate increase of mononuclear cells in the submucosa, increase in connective tissue in adults, widened spaces between the muscular bundles, and degrees of hyperplasia of the vessel walls, are not indicative of disease of the organ and occur far too frequently in appendices which have never given rise to any symptoms whatsoever. He has no hesitancy in declaring that a pathologic basis for chronic appendicitis does not exist. Those appendices in which changes are found to an unmistakable degree are found in persons who have had an acute attack but who no longer have complaints referable to the appendix. Hertzler's conclusions are perhaps a little overpositive and susceptible to some exceptions, but they are worthy of repetition here.

"1 Fibrotic changes in the appendix, no matter of what degree, are not attended by clinical symptoms

"2 The anatomic structure of appendices commonly removed under the diagnosis of chronic appendicitis shows no variation from the appendices of individuals suffering from no abdominal complaint whatsoever

"3 The minimal changes alleged to be present in cases of so called chronic appendicitis are wholly inadequate to explain the symptoms ascribed to them considered in the light of like changes in other organs of the body

"4 Mere alleged relief of symptoms after the removal of the appendix is not sufficient to prove that the appendix was the cause of the symptoms

"5 The vast majority of patients so operated upon do not even claim relief of their symptoms

"6 The symptoms alleged to be due to chronic appendicitis can be relieved by searching out the actual cause and by removing it relieving the patient without molesting the appendix

Heyd observed in his series that those patients in whom the appendix was removed for simple localized right sided pain were not cured but the patients that had appendectomy for symptoms referable to the upper abdomen and in whom there was no demonstrable pathology in either the gall bladder or the stomach were almost invariably cured. It was interesting to find that there was almost universally a history of a previous attack of pain which could be logically interpreted as an acute attack of appendicitis

Charles L. Gibson has recently reviewed a series of cases operated upon for chronic appendicitis in the New York Hospital and compared the results for the years 1913 to 1918 with those for the interval 1919 to 1923. He is inclined to attribute the obviously better results in the second series in great part to the substitution of picric acid for iodine as a disinfectant. However, a study of his paper makes one wonder whether more careful selection of cases may not have been a more important factor. At least if all surgeons would follow the rules which he has drawn up for selection of cases for operation the results should be excellent. These rules are as follows:

"1 A comprehensive and detailed history

"2 A complete and thorough physical examination, including all refinements of diagnosis

"3 Exercise caution in undertaking operation on women as compared to men

"4 Exercise caution, particularly in the more mature patients particularly women. In this class other lesions may coexist or may be mistaken for appendicitis

"5 Avoid the neurasthenics of any age or sex

"6 Exercise particular restraint when there is no clear and reliable history of well defined attacks particularly of localized pain accompanied by nausea or vomiting

"7 Make a good sized incision, and, even if a frankly pathologic appendix is found, look for other possible lesions

"8 If no obviously pathologic appendix is found, do not cease looking for other lesions until every other possibility has been exhausted, make a supplementary incision if necessary "

If we grant that there is much evidence against the so-called primary chronic appendicitis and against the justification for surgery in this type of case, the question naturally arises what is to become of the poor victim of this symptom complex? If he is to retain his appendix how may he be relieved? We must expect the answer from the internist or gastroenterologist, the neuropsychiatrist, the gynecologist, and the orthopedist. This group, except two, has attempted to formulate the answer in a symposium delivered not long ago before the Surgical Fortnightly Review, Boston.

Charles H. Lawrence presents the study of fifty cases showing symptoms of intermittent pain in the right lower quadrant, vomiting, nausea, and disturbance of function of the bowels over a period of a year or more. Twenty-one of these lost their appendices and of these twenty-one none was completely relieved by the operation. Seven obtained partial or temporary relief. The most constant symptom in the entire group was constipation. Twenty-seven showed definite cecal stasis with a more or less dilated cecum which in sixteen cases was definitely low or more or less fixed. Twelve showed stasis more or less throughout the colon, and two showed stasis in the ileum. Fifteen showed incompetent ileocecal valve, three diverticulae of the large bowel, three adhesions involving the bowel, and one patient showed a questionable duodenal ulcer on x-ray. In only one of the fifty cases was there a history of an attack that might be called acute appendicitis. The chronically inflamed appendix is probably only one evidence of a pathologic condition in the lower bowel. The condition consists of a mechanical obstruction to the proper evacuation of the intestine. Due to the inability of the intestine to empty itself properly, there results a low-grade inflammation not only of the appendix but of the cecal region and, in many cases, of a considerable portion of the large intestine. In many cases, if this low-grade inflammation is eliminated, removal of the appendix becomes unnecessary. The patient usually exhibits the picture of probable ptosis, constipation, and toxic absorption from the intestinal tract.

There is usually slight tenderness over the cecum without muscle spasm and with considerable gurgling on palpation. The cecum itself can often be felt. Pressure over the cecum often gives rise to nausea or epigastric discomfort, particularly in patients with incompetent ileocecal valve. Lawrence found that 34 per cent of his series showed headache or vertigo which was relieved when the stasis was overcome. Indican is often present in the urine.

Lawrence's outline of treatment consists first of elimination of the constipation and cecal stasis with laxatives in the beginning, if necessary, increased fluid and fruit intake, mineral oil, atropine and belladonna when indicated, a nonresidue diet with the avoidance of fats and dried foods and tonic setting-up exercises, particularly abdominal exercises. Abdominal supports should be avoided if possible. He finds that over 50 per cent of his series have been greatly improved by medical treatment.

Loring T Swain contributes an orthopedic discussion. He attributes the attacks in this type of case to the cecum failing to empty due to diet, associated colitis, increased sag, muscle fatigue, and general exhaustion from overwork. The problem is how to make the cecum empty and so remove the cause of irritation and to increase the general vitality. The treatment adopted consists in daily cecal massage. X ray studies have shown that manipulation of the cecum causes a mass movement of the whole lower bowel which quickly clears it of its contents in one big peristaltic push. Massage is therefore given directly after breakfast and after supper. It consists in a deep flat finger rotary movement beginning with the sigmoid and following the colon back to the cecum, the whole process taking about fifteen or twenty minutes. The massage is taken in two positions, one on the back and the other in a modified knee chest position. The second part of the régime consists in lying down after each meal with a pillow under the shoulders to hyperextend the chest and raise the diaphragm and all the organs below it. This is followed by a face position lasting for one half hour in order to change the position of the viscera. In order to prevent the loss of tone through sagging of the intestines when the patient is about his daily duties, all stoop shouldered attitudes are avoided and correct posture is insisted upon. In these exercises rib stretching and the abdominal muscle exercises play a part. Some patients are found to require the support of corsets or braces. A period of rest in bed for some weeks is requisite. Supports are discarded as correct posture is secured.

Edward L. Young, a surgeon, contributes similar ideas to the symposium. He finds that approximately one in five patients at the Massachusetts General Hospital in whom the diagnosis of urinary lithiasis was positively made had had from one to four previous abdominal operations in an attempt to relieve the condition. In the great majority the first operation had been an appendectomy.

He remarks that Deaver reports that out of 500 cases of chronic appendicitis operated upon 83.1 per cent were cured but 418 of these had had one or more attacks of acute appendicitis previously. Coller, of the University of Michigan found 89 per cent of 250 cases either cured or improved, but at the same time states that the prospect was not at all good without a definite acute attack in the past. He emphasizes that the fecal stream must be kept soft and all tendency to constipation avoided. Massage in the right iliac fossa while reclining in a tub of warm water is often very helpful as is also the medicine ball in certain cases. Correct posture is emphasized. In Young's discussion of the surgery of this condition, he pays much more attention to the cecum and ascending colon than to the appendix. He mentions two interesting cases. The first patient used abdominal massage for a year following operation, then tired of it and the symptoms returned. He again began massage, and they disappeared. This was repeated twice thus establishing the causal relation beyond question. The second patient had a return of symptoms after operation. He consulted three surgeons one of whom said he had gall bladder disease, another ulcer and a third adhesions. On postural treatment he was entirely relieved in less than three months without operation.

As Lawrence has well said, too often the appendix is only the innocent bystander in a bad neighborhood, and the surgeon an overzealous policeman who feels called upon to arrest some one, while the real culprit escapes

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—W T V

The Doctor and the Public

THERE is no denying the lack of information or the extent of the misinformation common to the public at large with regard to matters pertaining to health and disease

That it is extreme at times is commonly admitted, that it is the fertile soil upon which the seeds of quackery and pseudoscientific absurdity flourish is not always sufficiently emphasized or appreciated

We hear, nevertheless, something of the need for public education in these matters and see the application of various means and methods for the purpose

Some, at least, of the so called "health columns" of the daily press are of dubious value in that they are conducted carelessly, to give them the benefit of the doubt, others are the source of propaganda only, and still others fail of their purpose in some degree because it is one thing to have knowledge and still another to impart it to the other fellow And to an equal extent is this true of the radio Lectures and demonstrations are well enough in their way and are of value to those who attend them, but the horse cannot be forced to drink even though he be dragged to the stream

The most fallow field for instruction is right at hand and within the reach of every doctor—his patients

There is no physician who will not encounter the most gross ignorance and the most absurd ideas in those who consult him professionally This is his opportunity to dispel a certain amount of ignorance and to instill—even though it be in minor degree—a certain amount of correct information On the other hand, he may perpetuate and extend ignorance by careless, casual agreement with the patient that all his ills are due to "dyspepsia" and other similar pathologic amenities

It is the duty of every physician to be more interested in the prevention than in the cure of disease, for this is the aim of the profession toward which every endeavor is bent And ignorance and disease go hand in hand

If the people were taught how to *keep* well rather than labored with in the endeavor to *get* well, if the aims, efforts, and methods of preventive and prophylactic measures were popularly understood and popularly abetted, the prevention of disease would soon assume astounding proportions, as witness

what has already been done despite prejudice and opposition. That disease can ever be entirely eradicated is, of course, impossible. There will always be sufficient ignorance, at times expressed in the form of various 'antis' etc., and sufficient lack of interest which, together with natural effects and events, will suffice to continue disease as an entity. Nevertheless, a great deal can be done by persistent and consistent effort.

Nothing is more discouraging or more depressing to the scientific physician than to encounter cases of malignant disease or late syphilis, for example, when they are to all intents and purposes hopeless and to realize that this might have been and could have been avoided had only the patient come for aid at a propitious time or to realize further, that his reason for not doing so was because of a belief for example that as a popular physical culture" magazine teaches, syphilis is curable by a milk diet and cancer by the use of diet cancer pastes, or faith.

The doctor should always be ready willing and eager to seize every opportunity which presents itself to explain in *simple direct and understandable* phraseology the tenets of preventive medicine to make clear why certain methods such as vaccination, are efficacious for certain diseases and the like.

Nothing will ever be achieved by leaving it to the 'other fellow'.

The doctor should be the source of true and correct information, and his patients should be quick to appeal to him for this and confident of receiving it. If his own concepts and ideas are so muddled and so hazy as to render him incapable of imparting it then he is unfit for the practice of his profession. The least he can do is to refrain from careless terminology which tends to perpetuate if it does not inaugurate error.

The practice of medicine is a profession, and from certain angles, it has its business elements also, but it can never be dissociated from certain mandatory duties to the public. Its chief and greatest recompense comes at the end of the day's turmoil when and only if the doctor with his pipe in hand and perhaps, some favorite hook on his knee can say looking back over the day's work: I have done my best. I have omitted nothing which might help my most desperate case. I have done nothing which could harm the slightest one. My conscience is clear as to the patient who died.

So saying, he may face all his problems with confidence that greater nor more than this can anyone ask of him.

—R A K



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ON TO MINNEAPOLIS—SEVENTH ANNUAL CONVENTION

American Society of Clinical Pathologists

The members of the American Society of Clinical Pathologists are looking forward with eager anticipation to the next session at Minneapolis. The program bids fair to excel in interest and scientific import the magnificent record of previous conventions.

There has been a demand on the part of members to devote some consideration to the economic aspects of the field of clinical pathology. On Friday evening, June 8, there will be a Round Table Discussion, besides consideration of these matters at our business session.

The proximity of Minneapolis to Rochester will undoubtedly bring many of our members to the convention in order that they may profit by a trip to the Mayo Clinic.

The date of the meeting has been purposely placed as near to the American Medical Association Convention as possible in order to give our members opportunity to attend the big gathering. They will also be able to take advantage of the reduced transportation rates. By carrying the program over from the week end to Monday, the intervening Sunday can be profitably devoted to committee meetings, also the pleasant reunions among comrades in the common cause, the swapping of reminiscences of our early struggles, in the formation of new friendships and pleasant associations. The wives, too, and members of families of the Fellows will find in the convention a great opportunity both for sight seeing and social enjoyment.

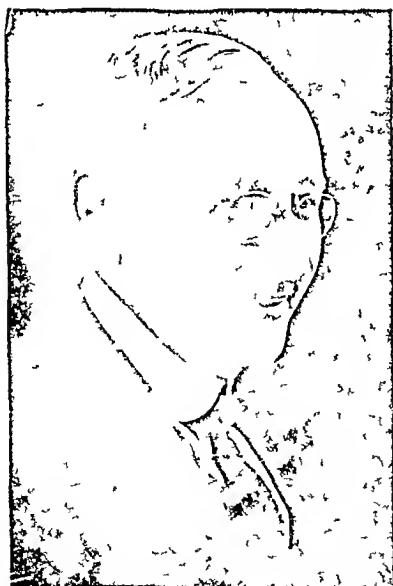
Below is a complete program containing the list of papers as approved by the Program Committee.



DR. H. J. CORPER
Denver, Colorado
Secretary Treasurer, pro tem
Chairman, Research Committee



DR. FRANK W. HARTMAN
Detroit, Michigan
President Elect



DR. PHILIP HILLKOWITZ
Denver, Colorado
Executive Committee
Chairman, Program Committee



DR. JOHN A. KOLMER
Philadelphia, Pennsylvania
Chairman, Executive and Publication
Committees

American Society of Clinical Pathologists Program

Of the Seventh Annual Convention

Minneapolis Minnesota

June 8 9 and 11 1928

FRIDAY MORNING JUNE 8 1928—9 A.M.

Call to Order

Short Business Session

Scientific Program

- The Interpretation of the Wassermann Test By B Markowitz M.D. Chicago Illinois
 Complement Preservative—A Practical Study By B W Rhams M.D. Fort Wayne Indiana
 The Interpretation of Borderline Allergic Reactions By Warren T Vaughan M.D. Richmond, Virginia
 The Vegetative Nervous System in Epilepsy By A M P Saunders M.D. Chicago Illinois
 Method for Measuring the Bactericidal Action of Whole Blood Against Gram Positive Cocci
 By William Thallmer M.D. and Charlotte Colwell A.B. Milwaukee Wisconsin
 Spectrophotometric Analysis of Blood Serum in Normal and Pathologic Conditions Study
 II By Charles Sheard Ph.D. T B Magath M.D. and A E Osterberg M.D.,
 Rochester Minnesota

FRIDAY AFTERNOON JUNE 8 1928—2 P.M.

- Some Tissue Culture Studies By F A Hecker M.D. Ottumwa Iowa
 Pathology of the Thyroid Gland By John W Gray M.D. Newark, N. J.
 Improvement in Technique and Results Made in Examining Microscopically by the Razor Section
 Method Two Thousand Malignant Tissues By B T Terry M.D., Rochester,
 Minnesota
 Further Observations with a New Method for Cultivating Tubercle Bacilli A Comparison
 with Guinea Pig Inoculation and Petroff's Method By H J Corper M.D., and
 Nao Uyei, Ph.D. Denver Colorado
 Some Observations Upon the Comparative Anatomy of Tuberculosis in Various Animals
 By Herbert Fox, M.D. Philadelphia, Pennsylvania
 A System of Sputum Analysis for Acid Fast Bacilli By Henry C Sweeney M.D. and Asya
 Stanichenko, A.B., Chicago, Illinois
 Tuberculous Endometritis By H L Reinhardt M.D., and Robert C Moore M.Sc. Columbus
 Ohio (By invitation)

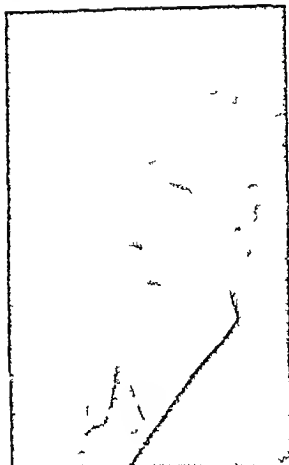
FRIDAY EVENING JUNE 8 1928—7 P.M.

Round Table Discussion

- The Postmortem Service at the University of Minnesota By E T Bell M.D. Minneapolis
 Minnesota
 The Young Clinical Pathologist By W G Gamble Jr M.D. Charleston South Carolina
 The Hospital Laboratory Director By J J Moore M.D. Chicago Ill.
 The State Laboratory By K D Graves M.D. Leesburg Virginia
 Membership By J H Black M.D. Dallas Texas

SATURDAY MORNING JUNE 9 1928—9 A.M.

- Retention of Urinary Constituents After Anastomosis of Urinary Bladder and Intestines
 By F W Hartman M.D. Detroit, Michigan
 The General Practitioner and the Early Diagnosis of Cancer By Wm Carpenter MacCarty,
 M.D., Rochester Minnesota
 The Sedimentation Time of the Blood in Jaundice By Nathan Rosenthal M.D. M I Blom
 stein M.D., M Rachmilewitz M.D., New York City



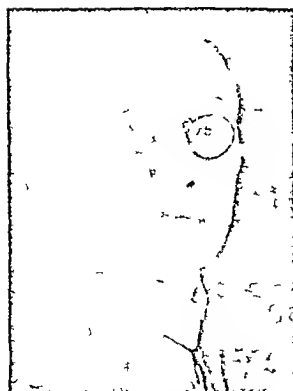
DR. W. W. COULTER
Houston, Texas
Incentive Committee



DR. HERMAN SPITZ
Nashville, Tennessee
Executive Committee



DR. F. E. SONDRÉN
New York City
Chairman,
Publication Committee
Executive Committee



DR. WM. G. EATON
Newark, New Jersey
Executive Committee



DR. GEORGE IVES
St. Louis, Missouri
Chairman, Board of Censors

- Studies in Sedimentation Time By Asher Yaguda M D, Newark N J
 Some Observations on the Mechanism of the Sedimentation Rate By O I Spahr M D
 and Robert A Moore M Sc Columbus Ohio
 A New Method of Colorimetry By Wm C Exton M D Newark N J
 The Chemistry and Cytology of Serous Fluids By Alvin C Food M D Guy Youngberg
 Ph D, and Vera Wetmore BS Buffalo New York
 Value of Nuclear Deviation (Schultze Classification) in Blood Examinations for Clinical
 Medicine By F W Niehaus M D Omaha Nebraska

SATURDAY AFTERNOON JUNE 9 1906—1 P M

- A New Method for Hemoglobin Determinations By Charles Skard Ph D and A H Sanford,
 M D Rochester Minnesota
 Pertinent Facts Concerning Hemoglobin By C F Frank M D Battle Creek Michigan
 The Specificity of Bacteria to the Bacteriolytic Action of Chemical With a Note on This
 Application to Chemotherapy By Rert A Kestly M D Washington D C
 Results in Various Diseases from the Elimination of Factors of Infection and the Use of Vaccine
 Prepared from Streptococci Having Exclusive Localizing Power By F C Loewen
 M D, and A C Nichol M D Rochester Minnesota
 Further Studies on Brucella Abortus in Man By A S Chordin M D and Mirjam
 Ahleson South Bend Indiana
 Lymphatic Leucemias and Mouth Infections By A S Lubutz M D Omaha Nebraska
 Examination of Blood for Malaria By Leon S Lippincott M D Natchez Mississippi
 Purpura Hemorrhagica with Report of Three Cases By Olin B Hunter M D Washington
 D C
 Estimating the Increment in Bacterial Load of Individuals Blood Produced by In-
 travenous Injection of Typhoid Vaccine By Charlotte Colwell AB and I J
 Yates M D (by invitation) Milwaukee Wisconsin (call by title)

SATURDAY EVENING JUNE 9 1906—1 P M

Annual Banquet

- Presidential Address By A H Sanford, M D Rochester Minnesota
 The Cults By Wm O Brien M D Minneapolis Minnesota
 Greetings from the American College of Surgeons By M T MacEneaney M D Chicago
 Illinois

MONDAY JUNE 11 1906—9 TO 12 AM AND 7 TO 12 M

Business Session

- Call to Order
 Reading of Minutes
 Unfinished Business
 Reports of Committees
 Executive Committee—Dr J A Kolmer Chairman Philadelphia Pa
 Public Relations Committee—Dr F E Sondern, Chairman New York
 Committee on Exhibits—Dr A C Broders Chairman Rochester Minn
 Publication Committee—Dr J A Kolmer, Chairman Philadelphia
 Research Committee—Dr H J Corper, Chairman Denver Colorado
 Committee on Registration of Technicians—Dr Kano Ikeda Chairman St Paul
 Minnesota
 Committee on Blood Clotting—Dr F W Hartman Chairman Detroit
 Service Bureau Committee—Dr R A Kilduffe Chairman Atlantic City
 Election of New Members
 New Business
 Nominations—Report of Nominating Committee
 Election of Officers
 Induction of Officers
 Adjournment



DR W F THOMSON
Beaumont, Texas
Board of Censors



DR. ROBERT A KILDUFFE
Atlantic City, N J
Board of Censors
Chairman, Service Bureau
Committee



DR H R MILS
Tampa, Florida
Board of Censors



DR REUBEN OTTENBERG
New York City
Board of Censors



DR. C H MANLOVE
Portland, Oregon
Board of Censors



DR A C BRODERS
Rochester, Minnesota
Chairman, Committee on
Exhibits



DR KANO IKEDA
St Paul, Minnesota
Chairman, Committee on Reg-
istration of Technicians

All the members of the American Society of Clinical Pathologists will mourn the untimely demise of the Secretary and one of the founders of the Society, Dr Ward T Burdick. His presence will be sadly missed at the next meeting. An obituary and biography will appear in a future issue of this Journal.

Dr Sanford, our President, has asked Dr H J Corper to act as Secretary Treasurer, *pro tem* until the next convention, when a successor to Dr Burdick will be elected. Address all correspondence to the American Society of Clinical Pathologists, Children's Hospital, Denver, Colorado.

AMERICAN ASSOCIATION FOR THE STUDY OF ALLERGY

Program

MORNING SESSION—JUNE 11, 1928

- 1 Castor Bean Dust Asthma
Karl D Figley, M D, Toledo, Ohio
- 2 Urticaria
Zella White Stewart, M D, Iowa City, Iowa
- 3 The Variability of Skin Reactions to Pollens
Harry L Huber, M D, Chicago, Ill
- 4 Asthma in Children Roentgen Study of the Chest Lantern Slide Demonstration
M Murray Peshkin, M D, New York City, N Y
- 5 Allergic Bronchitis
George L Waldbott, M D, Detroit, Mich

AFTERNOON SESSION

- 1 Presidential Address
Harry S Bernton, M D, Washington, D C
- 2 The Pathology of Asthma Autopsy Reports
Bernard Steinberg, M D, Toledo, Ohio
- 3 Incidence and Significance of Negative Skin Tests in Pollen Asthma in Infants and Young Children
I S Kahn, M D, San Antonio, Texas
- 4 Some Causes of Therapeutic Failure in Clinical Allergy
Warren T Vaughan, M D, Richmond, Va
- 5 The Mechanism of Negative Cutaneous Reactions in Hay Fever
Samuel Femberg, M D, Chicago, Ill
- 6 The Value of Phosphorus and Calcium in Bronchial Asthma, Hay Fever and Allied Diseases
A Study of 150 Cases
Alexander Sterling, M D, Philadelphia, Pa
- 7 Comparative Pollen Counts in Various Districts of the United States
O C Durham Indianapolis, Ind
- 8 Some Unusual Manifestations of Allergy
Samuel J Tish, M D, Chicago, Ill

MONDAY EVENING—BANQUET

Speaker of the evening

A Critical Review of the Mechanism and Terminology of Allergy

John A Kohner, M D, Professor of Pathology, University of Pennsylvania, Pa

MORNING SESSION—JUNE 12, 1928

- 1 Further Observations on the Use of Filtered Air in the Diagnosis and Treatment of Allergic Conditions
Milton B Cohen, M D, Cleveland, Ohio
 - 2 The Potential Asthmatic
F M Pottenger, M D, Monrovia, California
 - 3 Acquisition of Human Hypersensitiveness
Ray M Balvert, M D, Oklahoma City, Okla
- Election of officers for the ensuing year and a business meeting will be held following this session

The Journal of Laboratory and Clinical Medicine

VOL XIII

ST LOUIS MO JUNE 1928

No 9

CLINICAL AND EXPERIMENTAL

ON THE PATHOLOGY OF IRON*

By FREDERIC PROESCHER M.D. AND ALBERT S. ARIUSH M.D. AGNEW CALIF.

THE contents of this article are a continuation of our investigations *On the Pathology and Laboratory Diagnosis of Paresis*. Here we stated that a rapid demineralization of iron takes place in the brain due to disintegration of ganglion cells with phagocytosis of their contents. The iron from these cells was found about the blood vessels in their lumina and in the perivascular lymph spaces. It was also found in the spinal fluids having been ferried down from above by the large adventitial cells. In these it could be easily identified by any of the common stains for manganic iron.

No observations were made as to the exact nature and location of the iron within the cell nor were any extensive investigations carried out to ascertain whether or not similar processes take place on other tissues of the body. We therefore proceeded with these objects.

Moliseh¹ used solutions of potash to unmask the organic intracellular iron and showed firmly combined (masked) iron sometimes in the cell wall and sometimes in the cell contents and again in both.

Petit found nearly all the iron in barley to be combined with nuclein. These findings were from extraction experiments with acid alcohol. The affinity of dead nuclein for iron and certain dyes throws some doubt upon these findings.

Macallum² has thoroughly investigated the subject with non free reagents and extraction solutions of acid alcohol. Nothing to compare with this extensive research has been described since. As a stain he used acidified potassium ferrocyanide or ammonium hydrogen sulphide changing, some years later to the more sensitive and differential hematoxylin⁴. For further details on staining the reader is referred to these articles. Extracts therefrom pertaining to our work follow.

Unmasked (inorganic including albuminate) iron is retained by the chromatin. Action on blood is extremely slow ruling this out in all observed sections (p. 197). None of

From the Pathological Laboratory of Agnew State Hospital Agnew, Calif.
Received for publication November 21, 1927

the iron found in the nuclei is derived from the cytoplasm (p 199), though the diffusion may occur in the reverse direction during extraction (p 194) The greater part and sometimes the whole of the assimilated iron in the cells of higher animal life is held in the nuclei in the chromatin of which it is chiefly found It seems that the amount of iron demonstrated corresponds in all cases with the amount of chromatin present (p 205) Nucleolar elements also possess a trace of iron as shown by the Prussian blue reaction The nucleolus probably is not essentially formed of true chromatin in the cases studied The number, size, and shape of each are not constant, while not infrequently the central portion appears free from iron These bodies are always attached to the chromatin network (p 206) There is an inverse relation between the size of the nucleoli and the amount of chromatin in the network, and an examination of some nuclei in which the formation of the peripheral nucleoli has commenced and of those in which the development of these bodies is much more advanced, suggests that the latter are derived from the chromatin of the network (p 209) It would appear (from staining reactions) as if the iron compound undergoes a change in its transference from nucleus to cytoplasm (p 210) In the nuclei of all vegetable organisms the assimilated iron compounds are, on the whole, distributed as in the nuclei of the more highly developed animal forms (p 200) Mitotic figures in some sections were sharply defined through the iron revealed in the chromatin elements (p 211) In secreting cells a certain portion of the cytoplasm possesses masked iron At times it appears to occur in the zymogen granules and assumes different forms with the activity of the cell (p 223) Iron is contained in the chromatin of all cells (pp 228 and 268) Chromatin is the antecedent of hemoglobin (270) The two processes of respiration and assimilation, involving two activities of different natures, appear to Macallum to postulate the existence of two different iron compounds in the same nucleus, but Macallum finds no facts to indicate the occurrence of such (pp 270 271) Finally, returning to an earlier part of this paper we find "The peripheral nucleoli (in sections showing ova) appear to be formed at the nodal points of the chromatin network, but there is a possibility that these represent a pathologic condition I have, moreover, found that they are accompanied by examples of another condition which I regard as pathologic In the latter the nuclei were indistinct or disintegrated, their chromatin had disappeared, and the surrounding connective tissue, with its blood vessels and their red corpuscles even, gave in a few minutes, with warm ammonium hydrogen sulphide, an iron reaction frequently so deep as to obscure largely the details, while the tissues, a little further away from such samples, and other ova under exactly the same conditions of treatment with the reagent, gave no such reaction It may possibly be that the chromatin of such disintegrated ova furnished the iron observed thus diffused in the connective tissue and the blood vessels" (pp 210 211)

This last extract, which furnishes a basis or suggestion for our explanation and demonstration of the pathology of iron found in paresis, should logically have been incorporated in that article It was not known at the time of writing Since this work on paresis and in spite of the references quoted therein we have been able to demonstrate minute traces of pathologic deposits of inorganic iron in the brains of cases of senile dementia, encephalitis lethargica, and pellagra We believe that this is a necessary concomitant of cellular destruction wherever found The traces found were so extremely slight and widely distributed as to make any significant finding in the spinal fluid most unlikely It may be, in fact, that this iron was derived from impurities Continued experience in examining cases at the Agnew State Hospital has given results with our test as 100 per cent satisfactory

Further investigations by us have demonstrated that, for tissue work, hematoxylin is not a desirable stain It brings out too many extraneous elements and will demonstrate only inorganic iron Of the various acid-alcohols

we prefer sulphuric acid alcohol. It removes least rapidly the unmasked iron. Extractions made with this reagent and followed by the Berlin blue stain have demonstrated the following:

1 The ganglion cells of the brain contain iron centered in the nucleolus which body is unusually large. Some iron containing granules are shown in the nucleus. None occurs in the cytoplasm. The nucleolus generally shows a centrally placed unstained spot presumably the centrosome.

2 This pigment is stained by thiazin red (methylene azur) as is also cytoplasmic pigment. The latter is believed to contain iron in another form.³ The dye furnishes an excellent stain for nerve tissue in general.⁴ The fact that thiazin red will not stain chemically pure forms of inorganic iron in the test tube, as determined after many trials would indicate that at least a large part of the iron about the blood vessels and in the tissue of parietic brains is combined and therefore (supposedly) protected against cyanides and sulphides. Moreover cytoplasmic iron would seem to be masked in a different manner from that within the nucleus. We have also the following facts:

If the inorganic iron about the blood vessels of parietic brains be stained by the Berlin blue reaction and the tissue then extracted with acid alcohol no further iron reaction appears upon repeating application of the stain. Either the iron about the vessels is in a protected form but stainable with sulphides and cyanides, or those portions which are protected are very rapidly removed by the watery extraction methods. The latter seems the more probable.

3 The glial cells of parietic brains become when stained for masked iron, studded with granules varying in size from that of a pin head to that of a pin point* (as observed under a magnification of 1000x). The cells are very round. They may be seen to come up to a disintegrating ganglion cell until the two are contiguous. Two or three may feast upon the same cell at the same time. Whether or not living or functional cells are attached cannot of course be told with these methods. From the nucleus of the ganglion cell is consumed the surrounding cytoplasm and after engorgement the glial phagocytes will carry their burden to a blood vessel for deposition. Often they bunch in the pia where they may form strata several cells thick. Meanwhile the chromatin of the ganglion cell which is very slight outside the nucleolus seems to concentrate about the latter. This combined pigment appears in many cases, to burst or spread out into a spume or network which may some times be seen in the tissue after the remainder of the cell has disappeared. It is the swan song and perhaps represents an activity unknown since the birth of the person. The ganglion cells in their latabolism seem at times to throw off nucleoli as polar bodies are thrown off from maturing ova.

4 The perivascular cells seem to grow and swell as they accumulate iron splitting the layers of the vessel wall and eventually breaking into the perivascular space or (supposedly) the lumen of the vessel.

For a description of various glia see our article on parasites

5 Thiazin red and stains for inorganic iron show iron (presumably inorganic) in other parts of the body. No such extensive deposits as those found in paresis have ever been found except in liver, spleen, and marrow.

6 Actively growing cells in general seem oversupplied with masked iron. Very large cells in the liver were found to contain huge figures brought out by Macallum's method. In particular were the actively growing cells of carcinomas and melanosarcomas well stocked with masked iron. This finding is contrary to that of Martha Tracy⁷ in which iron was found to occur in tumors, both quantitatively and qualitatively, exactly as in normal cells of the same type. She also found that tumors react microscopically for iron either free or in the form of an albuminate only in cases where hemorrhages have occurred. Schwalbe⁸ found that cancer cells contain iron in a condition demonstrable by the Berlin blue reaction and occurring independently of hemorrhages. Wells⁹ states that iron in tumors varies from 0.013 per cent to 0.064 per cent (probably depending upon the amount of blood and nucleo-proteins) and Bell¹⁰ says that the blood contains (normally) about 3 gm of iron while the remainder of the body has from 1 to 3 gm of iron. For a 70 kg body these figures become percentages of 0.0014 to 0.0042 which means that if the figures are correct, tumor cells contain considerably more iron than the blood and still more as compared with the remaining tissue. Older portions of tumors did not show such excess of iron.

7 The changes in staining reaction make it appear that the iron goes through various stages of chemical combination, becoming less complex from the time when it is first thrown out of function from the masked or organic form until it disappears from about the blood vessel walls. It seems that the most simple inorganic forms are seldom reached until deposition in and about the vessel has occurred.

8 Lipoid bodies are suspected of containing iron because of their reaction to thiazin. Lipoidal preparations of colloidal iron have been prepared and give the same (light green) line with the dye.

9 In our article on nerve cell staining¹¹ a preparation was mentioned that brought out the glia cells very excellently and an attempt to repeat this result was promised. Such attempts have all failed. The variability in glial staining, combined with our findings in staining iron, indicate that the virtue did not lie in the particular stain used, but that the variability depends upon the iron complex content of the cells.

10 Blood of normal, febrile, and afebrile but sick persons was stained by the usual methods for demonstrating inorganic iron. Results were at first doubtful because of the bizarre figures obtained. In applying the Berlin blue reaction the white cells were found to be free from obviously stained granules. In the red cells, however, sometimes thread-like forms of black or blue-black were seen similar to those observed in the brain sections. There were also granular, arc-like, or ragged forms. These forms have doubtless been observed thousands of times by many microscopists and either ignored or passed by as artefacts. It was only because of their similarity to structures found in other cells (e.g., ganglion cell) and to other facts to appear later that our

attention was specially drawn to their study. They may be seen in unstained sections but are brought out better by certain dyes. Several possibilities were suggested. It may be iron absorbed upon the "disc" or contained within the disc, or the disc itself may have been partially stained or finally, the whole may have been an optical effect produced by the disc membrane. A special examination was made to decipher this phenomenon and will be described later herein.

11 Gravimetric determinations of the iron in parietic brains remain undone. Although the cells of highest iron content are destroyed and removed the difference in iron content while relatively great is actually so small that no definite results are to be anticipated from such labor.

DISCUSSION

General—Concerning those processes described for paresis specifically we have little further to add. It is to be noted however that the vicissitudes of the iron atom in its transference from ganglion cell to blood vessel are believed to result solely from the destruction of the cell and to be unrelated and unconnected, except in sequence with a specific virus. The latter directly or indirectly, appears to be the undisputable cause of the initial destruction.

This being the case it suggests that similar processes may be found generally. And this is so as shown by sections of various pathologic tissues. Study of paresis has the advantage that we find here a group of cells with high iron content rapid and widespread destruction and comparatively slow phagocytosis.

Perhaps the glial cells require considerable time in which to transform themselves into phagocytes.

The concentration of iron within the nucleoli of ganglion cells is interesting in view of the known lack of developmental activity of adult ganglion cells. When other tissues are observed an abundance of this iron may be found throughout the nucleus. The process causing death appears to be a spur to a latent or potential activity in the chromatin causing it to spread out into spireme or mitotic forms.

The exact chemical nature of the iron containing substances cannot be stated. It is not proper to assume that iron does not become inorganic until it leaves the cell. Macallum¹ has shown that iron both inorganic and masked occurs in all cells.* On the other hand we expect that the cytoplasmic iron if it actually does occur in lipoidal form may reach the vessels in a similarly lipoidal condition. Some of the iron about the vessels makes it appear (by its light green color with thiazin red) that this is actually the case. Finally, iron may be deposited in and about the vessel walls as the carbonate or phosphate this change being brought about by contact with the blood.

Fate of the Iron—One of the greatest interests lies in the final disposition of the destroyed iron. Katabolism follows anabolism throughout life and cellular destruction lends a supply of iron whose destination and means of

¹ A. P. Matthews late (according to Sollmann *Pharmacology*) that recent work throws doubt upon this as an invariable fact, and it seems to be true that in certain marine animals as medusae other metal e. g. vanadium may be substituted.

travel is hitherto unexplained. Inorganic iron has been found in egg yolk by Macallum¹¹ and in bone marrow by us and has been found by many in other hematopoietic organs. When we consider the various small depots of this element distributed about the body, as described above, we are caused to wonder if this is not all a part of one common process. Even the iron in the "Gitter" cells of the spinal fluid probably arrives in the blood stream eventually. The question is therefore in order: If iron is taken up from and delivered to bone marrow, liver, spleen, egg yolk and other hematopoietic organs by red cells, is it not possible that these erythrocytes are iron phagocytes or carriers and participate actively in the iron metabolism of the body as a whole as well as being passive carriers of an oxygen-transporting hemoglobin? To answer this question we offer the following considerations:

1. Macallum, as previously quoted in this paper, has found iron in these iron-distributing or iron-forming centers and in these areas only, to be con-

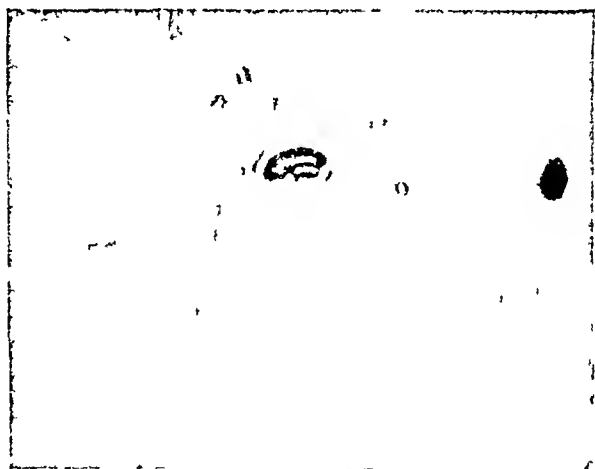


Fig 1—Iron body from the blood. Objective 45x, ocular 25x.

tained within the red corpuscles. It was apparently the same iron as that distributed locally in the pathologic tissues. The picture was obtained by staining for a few minutes with warm ammonium sulphide—a method that never stains the hemoglobin of the red cells. This iron was apparently inorganic.

2. The color of the cell is independent of the iron content so that no great fluctuation of color index would be expected in disease. Incidentally, we have no accurate means for determining the color-index to tell us if it does occur.

3. Unmasked (inorganic) iron is retained by the chromatin and chromatin is the antecedent of hemoglobin. Moreover, chromatin has a marked affinity for iron in various forms, even when occurring in dead nuclei.

4. In our sections showing iron in the lumina of blood vessels it has always been within the red cells or extracellular. Its appearance was generally similar to that in the blood smears previously described.

5 We have stained many smears of blood cells for inorganic iron using in general, acid potassium ferrocyanide. The number of the stained bodies observed within the red cells seems to agree quite well with the acuteness or probable destructive processes of the case as judged from clinical findings. Thus we found a tremendous amount in a case of pernicious anemia, very many in a case of bronchial pneumonia in a child and in a case of secondary anemia in a patient suffering from carcinoma of the cervix, somewhat less in cases of pyelitis, appendicitis, and another case of secondary anemia in a case of carcinoma of the cervix, less still in conditions with milder inflammation, and in only one of three cases of postnatal mothers were a few of these forms noted. Smears of normal blood gave no iron bodies at all or only a very occasional one. From such findings could be derived an iron index.

Though the great majority of iron forms discovered were within the red cells, here and there could be seen what appeared to be steps in the extrusion

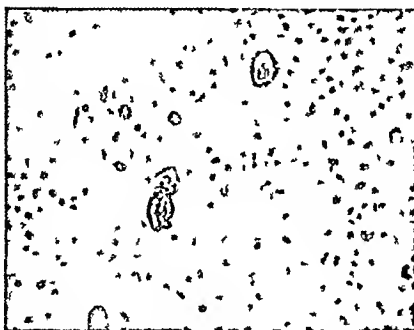


Fig. 2.—Iron body from paretic brain. Objective 4 x, ocular 5x.

of these bodies from the cell. Occasionally one sees them entirely outside of the red cells. Platelets moreover show granules of the same color in these smears.

Slides from pernicious anemia cases in remission were particularly interesting because the iron differed here from all other cases observed. It was more granular or fragmentary and in the case of immature red cells surrounded the outer membrane with a ragged margin. The strands were not heavy and black but were brown and fine. Very young normoblasts showed granules of what was apparently the same substance.

6 Brilliant cresyl blue and methyl green have been used with success in bringing out the iron bodies in the red cells. The former is the stain commonly used for the so-called reticular substance of the erythrocytes while the latter not only stains the iron in the tissue but also that in the test tube. This is true for both organic and inorganic iron. So far as we know, methyl green will stain only metals in organic form (including chromatin) or inorganic iron. The great quantities of stained material in the tissues examined

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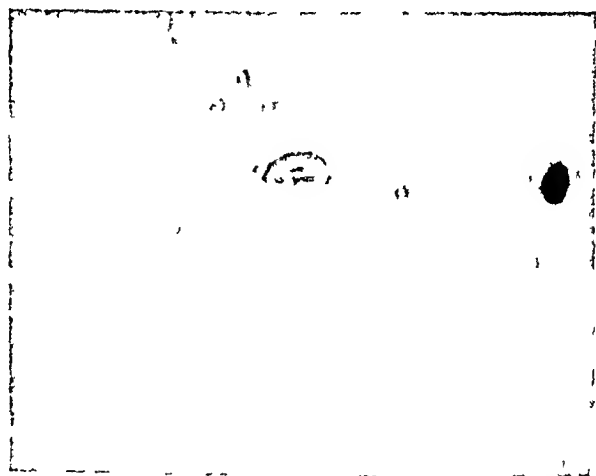


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3. Unmasked (inorganic) iron is retained by the chromatin and chromatin is the antecedent of hemoglobin. Moreover, chromatin has a marked affinity for iron in various forms, even when occurring in dead nuclei.

4. In our sections showing iron in the lumina of blood vessels it has always been within the red cells or extracellular. Its appearance was generally similar to that in the blood smears previously described.

Blood smears stain readily with methyl violet whereas with most other stains for inorganic iron there is no coloration of the cells or great periods of time are required e.g. hematoxylin. This is probably due in large part to the protective lipid membrane. The great value of methyl violet as a blood stain lies in its coloration of the red cells. Iron bodies previously referred to are violet (when heavy they appear black or unstained). The erythrocytes show within themselves zones of differing hues and colors while small cocclets or other forms sometimes appear within or without their substance corresponding presumably to the iron bodies. Some cells are a deep violet. Most of them show clear spaces (sometimes with a dark body of unknown character in the center). Color varying from pink to violet extends outwardly to the outer membrane where a deep violet rim exists. Distorted cell forms are also well shown. Obviously cells of different ages and structure have divided themselves into a new classification of erythrocytes. Our most striking pictures with this method were obtained in cases of pernicious anemia to which the above description more specifically refers. In secondary anemias the colors are more uniform both within the individual cell and within the cells as a group. The pictures obtained undoubtedly vary with the stage of the disease. Smears from a patient in the last stages of pernicious anemia showed a mottled pallor in the erythrocytes and extreme paucity of iron. The colors were more uniform in each cell though mottledly varied.

These stains have been applied to parietic brains and give excellent results in demonstrating the granules etc. discussed. We do not wish to infer however that only dyes belonging to the rosanilin or triamino triphenyl methane group will give a good reaction for iron. The fact is that using the perivascular iron deposits in the parietic brain as a criterion anilin dyes appear in general to be good iron stains. There are of course many exceptions. Neither do we wish to infer that all the staining power of some of the stains depends upon the iron content. The unspecificity of methyl violet has already been mentioned. This dye is very easily absorbed coloring even precipitated iron. Dyes not belonging to the rosanilin group and giving no iron reaction are exemplified by coelestinblau, alizarinbordeaux and naphthylazurin. All dyes used by us are Gieseler products.

7 When cells become more active as we have seen in leukemia itself and shall see further later they increase in iron content.

8 Stains of the bone marrow using ammonium sulphide acidified potassium ferrioxymide or hematoxylin show this tissue to be beautifully supplied with inorganic iron in all individuals. Granules occur diffusely among the various cells being particularly prominent in the eosinophiles. The young erythrocytes have their iron almost entirely scattered throughout the disc or upon the membrane. The heavy spleen forms are absent from this tissue. Amounts of iron in the different cells are extremely variable.

It would seem therefore that under normal conditions cells appearing in the blood stream transport comparatively little iron from the tissue to the hematopoietic organs except that of their original heritage. When pathological processes appear and iron from the cells obtains access to the blood the red cells scavenge such iron and transport it to the blood forming or pigment

collecting organs. Thus, they are found to serve an active function in iron traffic hitherto unknown. More accurate determinations of iron excretion in urine may confirm these views.

Very little has been said about the passage of iron in the reverse direction. This is, perhaps, because we were initially impressed by the phenomena of paresis in which obvious atrophy (prominent even macroscopically in old parietic brains) with disintegration and demineralization takes place. But we do not know that much of the iron seen about the blood vessels was not deposited there by red cells as part of a constructive process to the failing brain, this iron to be relayed to extravascular structures by wandering cells or in solution.* Thus Spatz, to whom we have referred so extensively in our previous article, may have been partially right in attributing the source of iron supply to red cells. Endothelial cells take up red cells or their fragments or cast off pigment, and even spontaneous hemorrhages may be an extreme example on the part of the tissue to increase its iron supply. Thus we may have both regenerative and degenerative iron in the tissue.

Although we are aware that there is an iron exchange within the body, no explanation has been offered of its mechanism which is compatible with its magnitude. If, as an index of this latter, we consider the pigment deposits and fluctuation in deposits in spleen and liver, it seems again that we must invoke the agency of the red cells. It has so far been impossible for us to tell, from examination of the blood smears, which of the iron is regenerative and which is degenerative. Perhaps comparison of specimens of the portal and vena cava inferior blood will solve this riddle.

If the iron observed about the vessels is inorganic, it is probably in some special physicochemical combination, for iron injected into rabbits could not be stained in smears from the blood shortly thereafter, using methods described above. It would seem, moreover, that the iron bodies contained in the red cells are related to the reticular bodies sought especially in cases of rapid blood destruction and regeneration. Brilliant cresyl blue will not stain inorganic iron in the test tube but, as noted, it does bring out or stain the iron within the red cell, while methyl green performs the latter function and at the same time stains all forms of iron—even that in the test tube. It is interesting to note that if any of this iron is combined with the hemoglobin we have what is chemically chlorophyll in the erythrocytes.

Malignant Growths—Malignant growths are, in our opinion, of high iron content. This is true at least of the rapidly growing tumor cells. We suspect, moreover, that the occasional successes reported in the treatment of carcinomas have been due either to the substitution of a metal (lead, gold, copper, etc.) for the iron of the cell or to a poisoning of this element (arsenic, infections, cautery).

Considerable quantities of iron may be thus withdrawn from the body. In three specimens of melanosarcoma examined by us, these deposits were tremendous—almost solid fields in places. It may be that iron which has functioned in these malignant cells becomes unfit to take up its place in the

*Hematoxylin has greater affinity for inorganic iron than methyl green. Double staining may be used. It brings out many minute dark granules in tissue nuclei and endothelium.

iron cycle or traffic. We have extracted thin sections with hydrochloric acid alcohol (the most rapidly extracting alcohol) for several hours. While the normal cells in the sections became entirely free from iron, that in the large cells of the tumor was but slightly affected at the end of this time. Not unlikely the atypical character of this iron is linked with the atypical character of the chromatin as indicated by the peculiar mitosis.

Sections of malignant tissue stained with methyl green show the iron most beautifully. While normal tissue which has very little iron is very slightly stained, carcinomatous cells stand out full and large and present a highly colored picture. Counterstaining enhances the value of the method as a differential stain for malignant tissue.

Cells in the immediate vicinity of the tumor cells have an inordinate amount of iron. Wherever connective tissue increases in activity, as about tumors or infections, more iron can be demonstrated than appears normally.

Research workers in Italy¹⁴ have recently cured malignant tumors in mice by repeated administration of sublethal doses of prussic acid. They believe that this is due to destruction of a ferment necessary to the cancer. We are inclined to agree with this conclusion and suggest that the ferment involved is iron, or an iron complex. With this idea in mind we are treating patients affected with malignant diseases with methyl green and cyanides (mercury cyanide at the present time). The work was started only recently and while we cannot report any brilliant cures so far, there has been considerable sloughing of necrotic tissue after local injection with excellent epithelization.

Pigment—Concerning melanin we can make no definite statements. It is generally recognized that the question of iron in this case is not settled. We can only say that in the cases of pigmented tumors mentioned above some of the pigment appeared green when stained with methyl green. That which retained its brown appearance shaded off gradually into other pigment which was distinctly green. Results obtained from a few smears of malarial parasites were inconclusive.

The formation of hemosiderin in the tissue after extensive hemorrhage is explained by these observations, in which the red cell deposits its inorganic iron to produce this pigment while the hemoglobin is resorbed or carried away without leading to the formation of organic iron.

CONCLUSION

Consideration of findings reported here has naturally led to endless speculation on our part, including interpretation of anemias and other diseases in terms of iron metabolism. Is there, for example, a limiting capacity for iron in each red cell which causes the cell to fragment when this limit is reached? Would iron then be an hemolysin and, if other cells take it up again, an anto hemolysin? Is pernicious anemia due to excessive ingress of iron from the intestines? Is the beneficial effect in malarial treatment of paresis obtained by destroying some red cells to stimulate the production of more and so clear

¹⁴According to Rous¹⁵ the initial state of normal red cell destruction consists in a fragmentation of the cells while they are yet in circulation. The finer particles are engulfed and dissolved by the phagocytic endothelial cells.

away the amino from the non-clad vessels and allow access of greater nutrition to the brain? Obviously, there is no end to such speculation.

But it seems that, if our interpretations be true, many new conceptions of pathologic and perhaps normal physiology must be formed. After many years of neglect we may come back to the study of non-appreciating in it a most active, important, and understandable substance.

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A CONTRIBUTION TO THE STUDY OF THE ERYTHROCYTE SEDIMENTATION REACTION*

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IN VIEW of the interest in the erythrocyte sedimentation reaction an investigation of the subject was begun in our clinical laboratory with the idea in mind of determining its value

Our work has been largely with cases of pulmonary tuberculosis. Although this paper may not present anything especially new on this subject yet it is felt that our findings are corroborative at least, of the results already reported. Our investigations seem to prove that the erythrocyte sedimentation reaction is not specific for tuberculosis that a parallelism exists between the severity of the disease and the increase of the sedimentation speed also that as the condition of the patient improves the sedimentation speed decreases, and that in some cases the phenomenon disappears before death.

Fahraeus¹ noted that when citrated blood was allowed to stand, the erythrocytes settled with different velocities in different pathologic and physiologic conditions. Since the publications of his observations in 1918 a great deal of confirmatory work has been done along this line especially in tuberculosis.

Popper and Kreindler believed the test to be valuable in diagnosis and prognosis not only of tuberculosis but in various other affections. They found that with progressing pulmonary tuberculosis an accelerated sedimentation rate obtained while in minimal and nonprogressive forms of the disease the rate remained within normal limits.

Morris² found a definite increase in the velocity of erythrocyte sedimentation in active pulmonary tuberculosis and believed that the test would be valuable in the determination of the degree of activity but not in the diagnosis of tuberculosis.

Cutler³ considers the sedimentation reaction as a more trustworthy aid in estimating the activity of a pulmonary tuberculous process than pulse rate, temperature or weight.

Glaus applied the test in the differential diagnosis of psychoses and found an increase in the speed of sedimentation in senile dementia, neurosyphilis, general paralysis, catatonia and immediately after epileptic seizures but no increase in neurasthenia, hysteria, paranoia nor manic depressive psychosis.

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RECORD OF TESTS DONE BY THE LINZENMEIER METHOD

DIAGNOSIS	NUMBER OF CASES	TIME IN MINUTES
Apparently normal individuals	134	680
Apparently arrested tuberculosis	88	820
TUBERCULOSIS		
Far advanced, rapidly progressive	69	44
Moderately advanced, slightly progressive	27	528
Far advanced, slightly progressive	65	175
Moderately advanced, rapidly progressive	2	36 5
Moderately advanced, nonprogressive	74	717
Minimal, nonprogressive	2	975
Hilum tuberculosis	1	110
Nonpulmonary tuberculosis, bone and joint	2	76 5
Tuberculous pleurisy with effusion	1	290
PULMONARY TUBERCULOSIS WITH TUBERCULOUS COMPLICATIONS		
Moderately advanced, pleurisy with effusion	2	27
Far advanced, pleurisy with effusion	2	28
Far advanced, laryngitis and intestinal tuberculosis	1	27
Moderately advanced, draining sinuses	1	115
Far advanced, rectal fistula	1	80
Far advanced, intestinal tuberculosis	3	85
PULMONARY TUBERCULOSIS WITH NON-TUBERCULOUS COMPLICATIONS		
Moderately advanced syphilis	7	223
Far advanced, syphilis	9	110
Moderately advanced, myocarditis	2	181
Far advanced, myocarditis	4	81
Moderately advanced, mitral insufficiency	3	600
Far advanced, mitral insufficiency	1	100
Moderately advanced, catarrhal jaundice, secondary anemia	1	50
Moderately advanced, psychoneurosis with suicidal tendencies	1	400
Far advanced, mitral stenosis, interstitial nephritis	1	28
Moderately advanced, arthritis	1	57
Far advanced, diabetes mellitus	1	30
Moderately advanced, myocarditis, cholecystitis	1	345
NONTUBERCULOUS DISEASES		
Otitis media	4	500
Pulmonary abscess	3	580
Spirochetal bronchitis	1	400
Myocarditis	6	550
Myocarditis, acute cystitis	1	100
Mitral regurgitation	2	147
Aortic regurgitation	1	300
Gun shot wound	1	600
Neurosis, type undetermined	1	330
Cardiac hypertrophy	3	1285
Abdominal adhesions	1	120
Myocarditis and chancreoid	1	350
Hyperthyroidism	3	490
Syphilis	32	420
Empyema following pneumonia	1	40
Chronic bronchitis	3	210
Acute gonorrheal urethritis	3	420
Chronic gonorrheal urethritis	1	90
Gonorrheal urethritis and epididymitis	1	500
Nontuberculous pleurisy with effusion	1	14
Chronic alcoholism	1	50
Pyloric ulcer, mitral regurgitation	1	600
Syphilis and catarrhal jaundice	1	50
Bronchial asthma	1	700
Gonorrhea and syphilis	1	180

DIAGNOSIS	NUMBER OF CASES	TIME IN MINUTES
Gonorrhea, syphilis and epilepsy	1	360
Suppurative adenitis	1	315
Lethargic encephalitis	1	1500
Chronic sinusitis and appendicitis	1	120
Chronic myocarditis interstitial nephritis	1	42
Catarrhal jaundice	1	480
Mitral stenosis	1	110
Chronic myocarditis, chronic bronchitis, drug addict	1	220
Pellagra	1	400
Myocarditis asthma, parenchymatous nephritis	1	5
Arteriosclerosis	1	800
Vincent's angina	1	480
Total Cases Examined	594	

Rayhonski's⁶ observations in tuberculosis indicate a parallelism between clinical findings and erythrocyte sedimentation, the more advanced the tuberculous process the greater the sedimentation speed.

Delhaye⁷ found that the sedimentation rate of fibroid types of tuberculosis approached the normal value while proliferative inflammatory types gave higher readings, the exudative types with severe tissue destruction very rapid sedimentation. He believed that a persistently high sedimentation rate implied a bad prognosis.

The observations recorded in this paper represent only a confirmatory study of the sedimentation reaction. Since a large number of patients are admitted annually to this institution for diagnosis and treatment (the majority being tuberculous) we had a splendid opportunity to test out the value of the reaction on several thousand patients.

METHOD

Blood for the test was taken from a prominent vein at the elbow and immediately mixed with 3.8 per cent sodium citrate solution in a proportion of four of blood to one of the citrate and thoroughly mixed.

Three methods were tested in the course of our study. First, that of Lanzenmeier.⁸ In this technique the time of sedimentation is taken as the standard. Small tubes, 0.5 mm in diameter and 6.5 cm long were used. One c.c. of the thoroughly mixed blood was placed in the tube and the height of the column of blood marked on the outside of the tube. 18 mm below this another mark was made. The time required for the erythrocytes to fall from the upper to the lower mark was the reading recorded for the test. Six hundred cases were tested by this method.

Second, the technique of Westergren was tried, using the modification described by Morris.⁹ In this case the citrated blood was drawn up in 0.2 c.c. graduated pipettes and the ends of the pipettes sealed with soft paraffin. Readings were taken after two hours and the percentage of sedimentation of the erythrocytes noted.

Third the graphic method described by Cutler was used with the exception of the amount of blood taken. Cutler described two methods, one in

RECORD OF CASES EXAMINED BY THE WESTERGRUN METHOD

DIAGNOSIS	NUMBER OF CASES	PER CENT OF SEDIMENTATION IN TWO HOURS
TUBERCULOSIS		
1 Pulmonary		
Far advanced, rapidly progressive	161	50.3
Far advanced, slightly progressive	92	43.4
Far advanced, nonprogressive	26	19.4
Moderately advanced, rapidly progressive	3	33.6
Moderately advanced, slightly progressive	32	16.2
Moderately advanced, nonprogressive	93	10.1
Minimal, rapidly progressive	1	42
Minimal, nonprogressive	3	5.2
Hilum tuberculosis	2	21
2 Nonpulmonary		
Tuberculous laryngitis	1	5
Bone and joint	11	48
Tuberculous orchitis and prostatitis	1	49
Renal tuberculosis	1	42
Tuberculous pleurisy	1	27
Glandular tuberculosis	1	63
3 Pulmonary Tuberculosis with Tuberculous Complications		
Moderately advanced with anal fistula	2	42.5
Moderately advanced, pleurisy with effusion	1	34
Far advanced, pleurisy with effusion	2	59
Far advanced, laryngitis, enteritis	1	60
Far advanced, laryngitis	2	55
Moderately advanced, draining sinuses	1	33
Far advanced, rectal fistula	1	32
Moderately advanced, spontaneous pneumothorax	1	2
Far advanced, intestinal lesions	1	27
4 Pulmonary Tuberculosis with Nontuberculous Complications		
Moderately advanced, syphilis	14	26
Far advanced, syphilis	19	39.8
Moderately advanced, myocarditis	3	36
Far advanced, myocarditis	4	38.2
Moderately advanced, mitral insufficiency	3	11.2
Far advanced, mitral insufficiency	1	23
Moderately advanced, catarrhal jaundice, secondary anemia	1	40
Moderately advanced, psychoneurosis with suicidal tendencies	1	14
Far advanced, mitral stenosis, interstitial nephritis	1	55
Moderately advanced, arthritis	1	35
Moderately advanced, chronic myocarditis, cholecystitis	1	17
Far advanced, diabetes mellitus	2	51.2
Far advanced, psychosis	1	64
Far advanced, gonorrhea	1	55
NONTUBERCULOUS DISEASES		
Otitis media	3	13
Pulmonary abscess	3	25
Spirochetal bronchitis	3	22.6
Myocarditis	7	15.6
Chancroid	4	13.5
Chronic myocarditis, acute cystitis	1	28
Myocarditis, syphilis	1	65
Mitral regurgitation	4	31.5
Smallpox	1	29
Paraplegia	1	26
Appendicitis	1	4
Aortic regurgitation	1	2
Gun shot wound	1	4
Neurosis, type undetermined	4	16.5
Cardiac hypertrophy	3	8
Abdominal adhesions	1	31

DIAGNOSIS	NUMBER OF CASES	PER CENT OF SEDIMENTATION IN TWO HOURS
Myocarditis chaneroid	3	11.3
Hyperthyroidism		9.8
Empyem following pneumonia	1	53
Follicular tonsillitis	1	19
Chronic bronchitis	1	20.9
Acute gonorrheal urethritis	8	9.1
Chronic gonorrheal urethritis	1	35
Gonorrheal urethritis epididymitis	1	22
Vincent's angina	2	10.5
Aneurysm of the aorta	2	45
Chronic bronchitis and sinusitis	1	26
Chronic sinusitis	1	32
Chronic bronchitis myocarditis	1	10
Nontuberculous pleurisy with effusion	1	15
Arthritis	1	10
Chronic alcoholism	1	43
Pyloric ulcer mitral regurgitation	1	4
Syphilis catarrhal jaundice	1	43
Bronchial asthma	1	4
Gonorrhea and syphilis	1	13
Gonorrhea syphilis epilepsy	1	30
Suppurative adenitis	1	16
Lethargic encephalitis	1	1
Chronic sinusitis appendicitis	1	18
Chronic myocarditis interstitial nephritis	1	46
Catarrhal jaundice	1	0
Mitral stenosis	1	27
Chronic myocarditis chronic bronchitis drug addict	1	20
Leishmania		29
Myocarditis asthma parenchymatous nephritis	1	40
Arteriosclerosis	1	2
Syphilis	99	10.9
Paralysis general	1	11
Gonorrheal arthritis	2	17
Duodenal ulcer	1	19
Sarcoma of the lung	1	60
Secondary anemia	4	60.1
Bronchiectasis following lobar pneumonia	1	16
Empyema	1	67
Diabetes mellitus	3	23
Mitral regurgitation	1	30
Apparently arrested tuberculosis	110	7.2
Myocarditis pericarditis aortic insufficiency secondary anemia	1	9.1
Amebic dysentery	1	68
Hookworm	1	10
Apparently normal individuals	290	6.8
Total number of cases examined	1009	

(These tables and charts represent work done only on male patients.)

which 5 cc of blood were used* and the other the so called finger puncture method⁹. In our work venous punctures were done because enough blood was taken at one time for erythrocyte sedimentation Kolmer complement fixation and Daranyi tests. Our method consists in drawing about 5 cc of blood from a vein and with a 1 cc pipette removing 0.4 cc and placing it in a small tube containing 0.1 cc of the sodium citrate solution. The mixture is then drawn up into 0.2 cc pipettes graduated in 0.01 cc divisions. These tubes are then sealed with soft paraffin and readings are taken every fifteen minutes up to two hours and the percentage of sedimentation noted. From the data obtained graphs are constructed.

DESCRIPTION OF CHARTS

Charts represent readings taken every fifteen minutes when tests were done by the modified Westergren method. Some of the curves show the average of the readings taken on more than one patient and others represent individual cases. The figures on the left side of the graph show the percentage of sedimentation, those on the bottom of the chart, the time interval at which readings were taken, that is after 15, 30, 45, 60, 75, 90, 105 and 120 minutes, respectively.

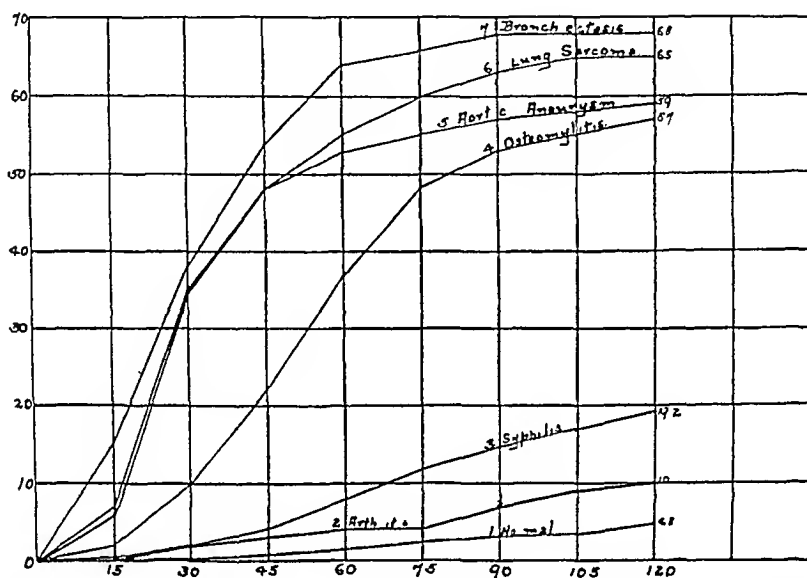


CHART I—Curve 1 at the bottom of the page is a composite one and represents the sedimentation rate of 50 nontuberculous apparently healthy individuals. Readings taken every fifteen minutes: 0, 4, 9, 15, 23, 31, 38, and 48 per cent of sedimentation respectively.

Curve 2: One case of arthritis. No tuberculosis. Readings: 0, 2, 3, 4, 7, 9, and 10 per cent of sedimentation.

Curve 3: A composite of 30 cases of syphilis. Nontuberculous. Readings: 0, 3, 19, 44, 81, 116, 145, 17, and 192 per cent of sedimentation.

Curve 4: One case of nontuberculous osteomyelitis. Readings: 2, 10, 22, 37, 48, 53, 55, and 57 per cent of sedimentation.

Curve 5: Aneurysm of the aorta. Kolmer complement-fixation test for syphilis weakly positive. Readings: 6, 35, 48, 53, 55, 57, and 59 per cent of sedimentation.

Curve 6: One case. Colored deceased. No tuberculosis. Lung sarcoma. Readings: 7, 35, 48, 55, 60, 63, 65, and 65 per cent of sedimentation.

Curve 7: One case. No tuberculosis. Patient has large amount of purulent sputum. No tubercle bacilli found. Diagnosis: Bronchiectasis. Readings: 15, 38, 54, 64, 66, 68, 68, and 68 per cent of sedimentation.

DISCUSSION

The Linzenmeier method was discontinued after making 600 tests. This technique is inconvenient for it requires almost constant attention until all of the erythrocytes have fallen to the given degree, 18 cm. Since some of the specimens of blood settled only after twenty-four hours, it meant that the test had to be observed during the night. One of the laboratory workers took the tests to her room and set an alarm clock for every hour in order to time the sedimentation with at least fair accuracy.

The Westergren method required far less time and gave more satisfactory results, for readings can be taken with greater accuracy in the pipettes than in the Linzenmeier tubes.

The Graphic method of Cutler required more attention than the Westergren, but gave better results. We used the 0.2 cc pipettes and took readings every fifteen minutes up to two hours and constructed graphs from the data obtained. This method proved to be practicable and is now used as routine on all patients entering this hospital. The graphs are very useful in follow up work, for the increase or decrease in the sedimentation rate can be seen at a glance.

With the Linzenmeier technique the shortest time of sedimentation was six minutes and the longest over twenty four hours. The rapidly progressive cases of tuberculosis gave the shortest sedimentation time while the chronic fibroid nonprogressive types of the disease approached the normal which Linzenmeier considered to be more than three hundred minutes.

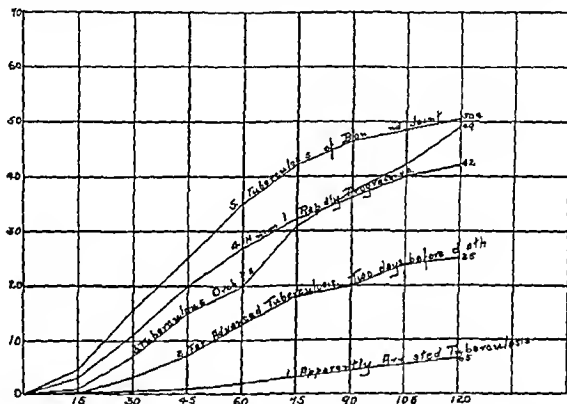


CHART II—Five classes of tuberculosis.

Curve 1. A composite of 1 apparently arrested cases of tuberculosis. Readings 0 1 0 6 1 3 1 4 3 5 6 and 6 5 per cent of sedimentation.

Curve 2. One case. Colored deceased. Blood was taken two days before death. Diagnosis: Far advanced pulmonary tuberculosis. Readings 0 3 13 18 20 4 and 5 per cent of sedimentation. This paradox was described by Cutler. He observed that in certain cases the erythrocyte sedimentation rate became slower just before death and the curve less steep. This may seem to come in this category.

Curve 3. One case. Nonpulmonary tuberculosis. Tuberculous orchitis and prostatitis. Readings 1 7 15 20 31 3 42 and 49 per cent of sedimentation.

Curve 4. One case of minimal pulmonary tuberculosis. Rapidly progressive with high temperature. Prognosis unfavorable. Readings 3 11 20 22 3 36 40 and 4 per cent of sedimentation.

Curve 5. Nonpulmonary tuberculosis. Tuberculosis of the bone and joints. Composite of five cases. Readings 4 6 15 6 5 1 34 8 4 46 4 48 6 and 50 4 per cent of sedimentation.

With the Westergren method the per cent of sedimentation for normal individuals was found to be 68 per cent in two hours. The greatest amount of sedimentation was 97 per cent in two hours in a case of secondary anemia (Erythrocytes 976,000 per cubic millimeter).

A study of the graphs shows a steep initial rise in the curve in rapidly progressive cases of tuberculosis as well as in other diseases which terminated fatally. There is only a slight rise in a normal individual's curve and in the chronic fibroid cases of tuberculosis. With increase in the severity

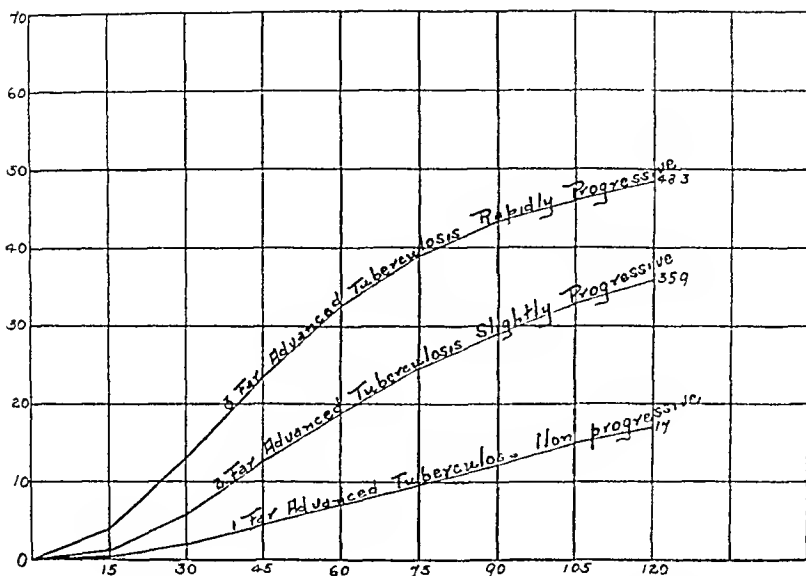


CHART III—This shows the effect of the activity of the tuberculous process on the sedimentation rate. All cases represented were classed as far advanced pulmonary tuberculosis. They were differentiated according to the severity of the disease.

Curve 1. A composite of seventeen far advanced nonprogressive cases of pulmonary tuberculosis. Prognosis good. Readings: 0, 1, 2, 4, 4, 7, 9, 7, 12, 1, 11, 8, and 17 per cent of sedimentation.

Curve 2. A composite of 23 slightly progressive cases. Prognosis fair. Readings: 1, 3, 5, 8, 12, 5, 18, 7, 24, 6, 29, 32, 8, and 35.9 per cent of sedimentation.

Curve 3. A composite of 30 rapidly progressive cases of far advanced tuberculosis. Prognosis guarded. Readings: 3, 8, 13, 23, 5, 32, 7, 39, 13, 3, 46, and 48.3 per cent of sedimentation.

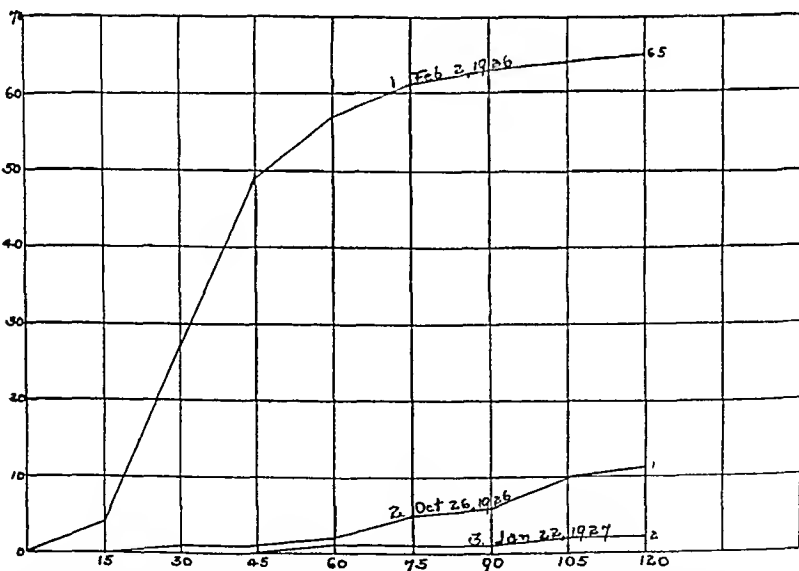


CHART IV—Represents the sedimentation rates of one patient before and after treatment. Diagnosis: Myocarditis, syphilis, and nephritis. Colored man aged sixty years.

Curve 1. at top of page shows the sedimentation rate on admission February 2, 1926. Readings: 4, 27, 49, 57, 61, 63, 64, and 65 per cent of sedimentation.

Curve 2. represents sedimentation rate after eight months treatment. (Mercury and dietetic). Blood taken October 26, 1926. Readings: 0, 1, 2, 5, 6, 6, 10, and 11 per cent.

Curve 3. Almost one year after admission. Readings: 0, 0, 0, 1, 1, 2, and 2 per cent of sedimentation.

of the disease there is a corresponding rise in the slope of the curve. With improvement a fall in the curve takes place.

A steep rise in the curve is also noted in early cases of tuberculosis of the rapidly progressive type. This seems to indicate that a rapid sedimentation rate is no index of the extent of the involvement but rather an indication of a lack of resistance.

CONCLUSIONS

- 1 The erythrocyte sedimentation reaction is not specific for tuberculosis.
- 2 A parallelism exists between the severity of the disease and the increase in the sedimentation rate.
- 3 With improvement in the condition of the patient the speed of sedimentation decreases, the opposite being true with increasing severity of the disease.
- 4 The phenomenon sometimes disappears shortly before death.
- 5 The sedimentation rate is not an index of the extent of the tuberculous involvement.

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THE REMISSIONS OF PERNICIOUS ANEMIA

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THE treatment of pernicious anemia has received much attention of late. The discussions almost invariably mention the proneness of the disease to remissions. There are, however, characteristics of this proneness to remissions which have not been emphasized apparently.

Data on 524 fatal cases of pernicious anemia which have been collected by Cabot¹ state that the number of remissions was as follows: 296 one remission, 118 two remissions, 65 three remissions, 21 four remissions, and 24 five remissions. These data indicate that practically no one dies without at least one remission (such cases have been reported but apparently are quite rare) and that there is no tendency for the progressiveness of the disease to change. The progressiveness remains a constant when measured by the chance of death after any one remission. This constancy of the progressiveness can be made evident by assuming that the chance of having another remission is one half. The series on this assumption would then be 262, 131, 66, 33, and 16. When the two series are arranged side by side, it is apparent that the parallelism between

the two sets of figures, one biologic and the other a hypothetic geometric series, is so striking that it must command attention as something more than a coincidence at least

NUMBER OF REMISSIONS	ACTUAL	HYPOTHETIC
1	296	262
2	118	131
3	65	66
4	21	33
5	24	16
Total	524	

If the duration of a remission plus the time between remissions is a constant, it follows as a consequence of the above one-half chance law that the number of surviving pernicious anemia patients in any random group will have had or be in remissions as follows 50 per cent one remission, 25 per cent two remissions, 12½ per cent three remissions, etc Data for the rough check on a "cross-section" of a group of survivors are afforded by a recently published² series of 42 surviving cases They had had attacks as follows 20 one attack, 13 two attacks, 4 three attacks, 4 four attacks, and 1 five attacks According to the one-half chance law the figures should be 21, 11, 6, 3, and 1 A comparison of the two series shows that the check is very good in spite of the fact that the total number of individuals is rather small for such an analysis

NUMBER OF ATTACKS	ACTUAL	HYPOTHETIC
1	20	21
2	13	11
3	4	6
4	4	3
5	1	1
Total	42	

It is frequently stated that subsequent attacks of most diseases which recur after remissions become progressively lighter and "finally the disease wears itself out" Such does not seem to be the case with pernicious anemia The following statements appear to be justified

1 Pernicious anemia is a disease in which very few die in the first attack
 2 The remissions follow a strict law of probability, the chance of having another remission is one-half

3 The average duration of a remission plus the time interval between remissions (= duration of an attack) appears to be a constant

These facts have been formulated and prepared for publication at the request of my teacher, Dr Kamil Schulhof

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HAY FEVER AND ASTHMA CAUSED BY THE POLLEN OF THE PAPER MULBERRY (PAPYRUS PAPHYRIFERA KUNTZE)

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THE list of pollens which are regarded as causative agents in hay fever and asthma is an ever increasing one. There is no record in the literature, however, of any case in which the pollen of the Paper Mulberry (*Papyrus papyrifera* Kuntze) has been described as the etiologic factor. Therefore the three patients, herein reported, are of unusual interest and merit a detailed description.

CLINICAL DISCUSSION

CASE I—Male, aged twenty-four years

On May 11, 1926, this patient first presented himself for relief of acute hay fever and asthma. His symptoms appeared on April 30, eleven days prior to his visit, and were becoming progressively worse. Paroxysmal sneezing, stuffiness of nose, dyspnea, tightness of chest, and wheezing had incapacitated him. His condition at the time of his first visit did not permit of any extensive examination. Nevertheless, a subcutaneous injection of five pollen units of Timothy pollen extract was given in the right upper arm. The purpose of this procedure was twofold: first, to aid in the diagnosis of the type of hay fever, and second, to initiate a possible course of seasonal therapy. The absence of any local reaction to the injection of Timothy pollen extract indicated that the grass pollens were not responsible for the disease. It is noteworthy that the date of onset of symptoms in this patient coincides with the period of pollination of the late blooming trees and with the pollination of the early blooming grasses. Very sensitive venereal cases will exhibit symptoms in the latter part of April at which time *Poa annua*, the first of the grasses to bloom in the District of Columbia, sheds its pollen.

Palliative measures were then prescribed for the patient. On the following day, May 12, 1926, a series of thirty-two cutaneous tests were performed with pollens in powder form and with a few epidermal proteins. The list comprised the following:

On the left lower arm

Wormseed	Sheep Sorrel
Timothy	Goose Feather
Rabbit Hair	Orchard Grass
Black Walnut	Chicken Feather
Short Ragweed	English Plantain
Elm	Dog Hair
June Grass	Sycamore
White Oak	Pigweed

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On the right lower arm

Black Ash	Box Elder
Butternut	Blue Beech
Beechnut	Hickory
Swamp Maple	White Birch
Cherry Birch	Honey Locust
Willow	Pussy Willow
White Mulberry	Paper Mulberry
Black Gum	White Poplar

In summary, four epidermal proteins, twenty tree pollens, six pollens of vernal hay fever plants, and two pollens of autumnal hay fever plants were utilized in establishing a diagnosis. Of this number, the pollens of the White Mulberry and of the Paper Mulberry presented typical urticarial wheals with zones of irritation at the site of the scratch marks. The findings proved as interesting as they were unexpected.

In 1922, I began a survey of the wind pollinated plants in the District of Columbia¹ and, on May 1 of that year, I collected the pollens of the trees of White Mulberry and Paper Mulberry. Four years later during the course of innumerable tests, these pollens gave positive reactions for the first time. The patient who responded positively to these tests has lived within three and one half blocks of the very trees from which the pollens had been obtained.

On the occasion of the patient's third visit on May 13, 1926 confirmatory tests by the cutaneous method were performed. On the right forearm, the pollens of the Paper Mulberry and of the White Mulberry were applied to scratch marks, and on the left forearm, the pollen of the Mock Orange was similarly employed. Each of the three test pollens yielded a positive reaction. The reaction, however, caused by the pollen of the Paper Mulberry came up promptly. It was most marked and the intense local irritation persisted longer than that of the other two pollens. These three positive reactions furnish a striking example of "group reaction."

It is noteworthy that in the Flora of the District of Columbia² and vicinity, Hitchcock and Standley have arranged and numbered the plants consecutively according to generic relationship. The Moraceae, the Mulberry family, constitute Group No. 40 which has the following subdivisions:

1 Toxylon pomiferum	Mock Orange
2 Papyrus papyrifera	Paper Mulberry
3 Morus	
a Morus alba	White Mulberry
b Morus rubra	Red Mulberry

Accordingly, the diagnosis of greater sensitiveness to the pollen of the Paper Mulberry was warranted by the findings and a basis for specific therapy was established. Difficulty, however, was encountered because I had no extract of the Paper Mulberry pollen available. It had seemed unlikely that a need should ever arise for the extract.

On May 14, 1926, an intradermal test was performed with two pollen units of an extract of Mock Orange which had been prepared a few months previously. The resulting wheal measured fourteen millimeters and the

areola thirty millimeters in diameter. The hope was entertained that desensitization might be effected with an extract derived from another member of the same family group. Schuppegrull has advocated this principle in his immunologic classification of common hay fever plants.³ Accordingly, on May 15 1926 five pollen units of an extract of Mock Orange were injected subcutaneously into the upper arm. There was no local reaction. At this point, it is well to anticipate and to record an observation which establishes a diagnostic procedure of importance. On February 12, 1927 five pollen units of an extract of the pollen of the Paper Mulberry were administered subcutaneously in the same patient at the beginning of a subsequent course of preseasonal treatments. The local reaction consisted of a swelling of the upper arm which measured ten centimeters in greatest diameter. There was marked congestion and also sensations of heat and of itching of the part involved. Moreover mild hay fever symptoms persisted for two days after the injection of the five pollen units of the Paper Mulberry extract.

In a previous communication⁴ I have shown that "the subcutaneous reaction to pollen protein is a more critical and specific index of mucous membrane sensitiveness than the cutaneous or intracutaneous tests." Kahn and Grothaus are in accord with this view. Despite the positive cutaneous and intracutaneous reactions obtained with the pollen of the Mock Orange it seemed inadvisable to use its extract in treatment of the patient. The pollen of the Mock Orange it will be recalled failed to provoke a local reaction on subcutaneous injection, a fact which militated against its use. Moreover, the number of trees of Mock Orange in the District is relatively insignificant.

Meanwhile, the acute symptoms of hay fever and of asthma persisted, ameliorated to some extent by the use of palliatives. Under the circumstances, the daily subcutaneous injection of small doses of pollen extract, as recommended by Vaughan for the treatment of the acute attack could not be carried out. As stated before there was no extract of the pollen of the Paper Mulberry available. For the same reason the relief of acute symptoms by intradermal injection of pollen extract the method of Phillips⁵ was denied. One alternative remained which happily proved successful.

My method consisted of the daily application of the pollen powder to a scratch mark on the skin of the forearm on which a drop of dilute sodium hydrate solution was placed. The procedure was comparable to the cutaneous test as ordinarily performed. The record indicates that fourteen cutaneous applications of the pollen of the Paper Mulberry were made in the interval from May 17 1926 to June 5 1926, inclusive. The sudden improvement in symptoms was most gratifying. On May 18 1926 the patient reports "Marked local reaction and intense itching of arm. Feels fine." On the day following he reported "No hay fever and no asthma." On May 22 1926, there were no hay fever symptoms. Only a little wheezing in the morning."

It may be argued that the termination of symptoms may have been due to the cessation of pollination and not to treatment. That, of course, is a possibility. Untreated cases of hay fever however, show a gradual cessation

of symptoms toward the end of the season and not the abrupt cessation which has been so evident in this patient. The change in the clinical picture was similar to the one usually encountered when administering the specific treatment during the attack of hay fever in accordance with Vaughan's directions. In fact, the experience of my patient lends support to Vaughan's explanation of the mechanism of seasonal therapy. The author states, "During the pollen season, the nasal mucosa is bearing the brunt of the allergic reaction. The administration of pollen elsewhere, as through the skin (in my case, on the skin), would theoretically distribute the reaction throughout the other tissues, thereby relieving to some extent the intensity of the local reaction."

The patient was seen next on January 27, 1927, seven and one-half months after the termination of his seasonal treatment. He reported that he had been singularly free of nasal catarrh during the fall and winter months. In the interim, extracts of the pollens of the Paper Mulberry and of the White Mulberry had been prepared in anticipation of the course of preseasonal treatments which the patient had requested. On two different occasions, entaneous tests were performed with these extracts in the dilution of 1:100 and 1:1000. In each instance, the extract of the Paper Mulberry pollen yielded the larger reaction and the persistence of induration for twenty-four hours around the scratch mark was particularly noticeable. The subcutaneous injection of five pollen units of the White Mulberry extract gave rise to a swelling two and a half centimeters in diameter which was surrounded by a zone of redness and accompanied by some itching. On February 12, 1927, five days later, five pollen units of the Paper Mulberry extract were administered subcutaneously. As previously stated, the local reaction to this extract measured ten centimeters in diameter, and hay fever symptoms ensued for forty-eight hours after the injection. Five pollen units of the extract of Mock Orange did not give rise to any local reaction whatsoever. In virtue of the above findings, the diagnosis of marked sensitivity to the pollen of the Paper Mulberry and of lesser sensitivity to the pollen of the White Mulberry was warranted.

The course of preseasonal treatments was begun on February 14, 1927. The quantity of extract was gradually increased from the initial dose of five pollen units to the terminal dose of one thousand pollen units which was administered on April 14, 1927. As result of the treatments, the patient reports, "I have been improved 99 per cent. I have had no asthma, and only a slight discharge from the nose for two days during the entire season."

Other facts pertaining to the history of this patient are now presented to make the record complete. He is a native of Greece, having immigrated to this country at the age of eleven. When first seen in 1926, he was twenty-four years old and was entering upon his third season of hay fever. The disease was becoming progressively worse. In 1924, the symptoms extended from April 7 to June 15, in 1925, the season was of one month's duration from April 20 to May 20. In 1926, hay fever appeared on April 30, and two days later, asthmatic symptoms complicated the disease for the first time and proved disabling. He has two brothers who are subject to the autumnal

type of hay fever and two sisters who have been asthmatics for many years. His father, now deceased, had also been a sufferer from asthma.

It is noteworthy that this patient as a boy had assisted in the care of silk worms. The silk industry is essentially a home industry in Greece, and its success is dependent upon furnishing adequate food in the form of mulberry leaves to the silk worm in the feeding season. Chater³ states, "The silk worm has a voracious appetite for a creature three and one half inches long, and during its brief life of thirty days it consumes six times its own weight in mulberry leaves." Exposure to the pollen of the Mulberry during the boyhood of my patient has undoubtedly furnished the sensitizing agent to one who had inherited a predisposition toward sensitization.

CASE 2—Female white, aged fifty seven years

This patient has been a victim of hay fever for fifteen years. With increase in duration of the disease there has been an increase in its severity. The date of onset is April 15 and the date of termination the latter part of May or early June. The average duration of her disability is six weeks. The symptoms are typical of the disease. The eye symptoms, however, are particularly aggravated in her case. The eyelids become swollen obscuring vision. Moreover, the urinary bladder shows an extreme irritability during the season, making frequent urination necessary.

She was first seen by me on March 10, 1927. A study of her case similar in scope to the one described above, revealed a sensitiveness to the pollen of the Paper Mulberry. Accordingly preseasonal treatment was initiated on March 14, 1927. This patient proved unusually sensitive to the pollen extract. The third injection of eleven pollen units provoked a constitutional reaction of moderate severity. On April 14, 1927, after one month of treatment, the dosage reached only fifty pollen units. Severe hay fever symptoms on April 15 marked the usual beginning of her seasonal distress. From April 16 to May 24, 1927, seasonal therapy was instituted at twenty-four hour intervals at first and at forty-eight hour intervals later in the course. The terminal dose consisted of two hundred and forty pollen units.

Despite the patient's extreme sensitiveness to pollen extract and inadequate protection, as judged by the accepted standard, the extent of relief from her wonted disabilities may be surmised from the following report: "I realize that your treatments were not started in time due to my delay, but I can and do say that they have been a wonderful help to me. The treatment did not entirely prevent the cold * * *, but it certainly greatly reduced my suffering. As a matter of fact there were only three days during the whole period in which itching of the eyes and swelling occurred. I had no asthma as in former years although I was troubled with a severe cough * * *. I certainly feel that your treatment * * * has given me the only relief I have had and this to a considerable degree."

CASE 3—Female, colored, aged forty two years

This patient registered at my clinic at the Emergency Hospital in Washington, D. C., on March 11, 1927. In her history she stated that she had been a sufferer from hay fever and asthma for a period of ten years. The

duration of symptoms extended from April 25 to June 1. Cutaneous and subcutaneous testing indicated that she was sensitive to the pollen of the Paper Mulberry. The patient was a domestic servant by occupation. Because of her inability to leave her work, she was irregular in attendance. She received only ten injections of pollen extract. The first dose of five pollen units was administered on April 1, 1927, and the terminal dose of forty pollen units on May 13, 1927. Each injection provoked a local swelling four inches in diameter.

The following selected clinical notes in her case are of interest. On April 21, 1927, she experienced for the first time itching of mucous membranes and slight wheezing. April 25, "No trouble at all since last visit." May 9, "Some itching of eyes and running of nose. Sneezes four times daily instead of the usual fifty or sixty times." On a subsequent visit on June 6, 1927, she reported that she had not lost a single day from her work, whereas during other hay fever seasons, she was unable to work because of her asthmatic symptoms.

CLINICAL SUMMARY

CASE NO.	AGE	FAMILY HISTORY	DURATION OF DISEASE	PERIOD OF SYMPTOMS	REMARKS
1	24 M	Positive	3 years	April 16 (Average) to middle of May or of June	Severe asthma
2	57 F	Negative	15 years	April 15 to early June	Moderately severe asthma. Swelling of eyelids. Urinary bladder irritability.
3	42 F	Doubtful	10 years	April 25 to June 1	Severe asthma

BOTANICAL CONSIDERATIONS

The Paper Mulberry (*Papirus papyrifera* Kuntze) is a tree native to Eastern Asia. It has been extensively cultivated in the Orient because of the use of its bark in paper making.⁹ It had been cultivated in this country as early as 1750. Its range of distribution extends from New York to Florida, and it is also found in Missouri. The smooth grey bark and long staminate catkins are among the distinguishing features.

In the City of Washington, whose beauty is enhanced by its tree-lined streets, there are many specimens of the Paper Mulberry. Curiously enough, these trees are rarely found on the streets. They seem to prefer vacant lots and yards and abound in alleys. This is especially true of the older sections of the city. Moreover, the staminate trees predominate, a pistillate tree is a rarity. New growth arises from suckers.

A survey of the trees with particular reference to the Paper Mulberry in the neighborhood of my patients readily disclosed the source of their disabilities.

Case 1 was domiciled in one city block and was employed in an adjoining block. Six paper Mulberry trees were found in the alley of one block.

An equal number of Paper Mulberry trees, two White Mulberry trees, and one Mock Orange tree grew in the other block. In fact, his bedroom window overlooked the two White Mulberry trees.

Situated in the alley within half a block of Case 2, one Paper Mulberry reared above the house tops, whereas two specimens of the trees occupied positions in front yards within the half block in which Case 3 dwelt. The three patients lived in sections of the city remote from one another.

A review of the literature of the eleven commercial establishments, ~~located~~ raised by the United States Public Health Service to manufacture pollen extracts, proved of interest. One firm listed the dry pollen of the Red Mulberry (*Morus rubra*), and one other firm had available the pollen extract of the Black Mulberry (*Morus nigra*). The New England States, the Eastern States and the Southern States were cited as the territory within which the Black Mulberry might be found as a cause of early spring hay fever. No mention, however, was made of the Paper Mulberry.

A similar omission was noted in a search of the publications of students of hay fever. Scheppegegrill in an early paper which appeared in 1917, includes the White Mulberry (*Morus alba*) in a partial list of plants tested for hay fever reaction at the Biological Laboratory of the American Hay Fever Prevention Association. The following reference to the White Mulberry is quoted: "Pollen grains, smooth spherical, nineteen microns in diameter. Hay fever reaction negative. Pollination, wind. Amount of pollen, abundant. Season of bloom, May. Geographic distribution: Maine and Ontario to Florida. Remarks, harmless in hay fever."¹⁰ Furthermore, Scheppegegrill does not include any member of the Mulberry family in his classic paper on the "Seasons, Causes and Geographic Distribution of Hay Fever and Hay Fever Resorts in the United States" published in 1920.¹¹ Neither was there any reference made to the family under the heading "The Trees in Hay Fever," in the author's¹² textbook nor in another important contribution which was published one year later, in 1923.

It is noteworthy that authors who discuss the botany of their respective regions omit mention of the Mulberry family. Thus, Walker¹³ of Boston, Spivaacke¹⁴ of New York, and Kahn¹⁵ of Texas, do not include the pollen of the Mulberry family in the list of causes of hay fever or of pollen asthma. Likewise, Duke and Durham¹⁶ fail to record the Mulberry family in their botanic survey of Kansas City, Missouri and neighboring rural districts. The state of Missouri according to Rehder,¹⁷ represents a midwestern habitat of the Paper Mulberry. Nevertheless, other representatives of the Mulberry family have been reported on the Pacific Coast. Hall, in his "Hay Fever Plants of California,"¹⁸ makes the following interesting comment: "*Morus alba*, White Mulberry. Mulberry Fam. This and a few other species, especially *M. nigra*, the Black Mulberry, are grown to a limited extent near towns. Probably never a cause of hay fever. Spring. Summer." Moreover, Piness¹⁹ has not reported any case of hay fever caused by the Mulberry family from his vast clinical experience.

SUMMARY

The pollen of the Paper Mulberry (*Papyrus papyrifera* Kuntze) is herein recorded as a cause of Spring hay fever and pollen asthma

In addition, the foregoing study emphasizes a few fundamental considerations of hay fever which are frequently overlooked

1 Cutaneous sensitiveness to pollen does not always indicate a mucous membrane sensitiveness to the same pollen

2 The subcutaneous reaction to pollen protein is a more critical and specific index of mucous membrane sensitiveness

3 The relief of nasal symptoms during the hay fever season by the frequent subcutaneous, intracutaneous, or cutaneous application of pollen extracts or of pollen powder results from the participation of other tissues in the reaction to a toxic irritant

4 Regional field surveys are of paramount importance One hour in the field is worth three hours in the library

It is my pleasure to acknowledge the invaluable assistance, generously accorded me, by Mr Homer C Skeels of the United States Department of Agriculture

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ON BACTERIOPHAGE FROM NORMAL STOOL CULTURES*

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IN THE literature on bacteriophage several reports may be found of attempts to isolate bacteriophage from stool cultures. A number of methods have been employed. Mallman¹ filtered broth cultures and tested the filtrates on the homologous organisms. Gildemeister and Herzberg² and Burgers and Bachmann³ triturated the bacteria and extracted the bacterial juices under pressure. It is reported by d Herelle⁴ that Pondman⁵ filtered broth cultures and tested the filtrates for the presence of bacteriophage. Pondman also dissolved the cultures with trypsin or ptyocyanase, and in other experiments, substituted heating for filtration. Flu⁶ examined many strains of the typhoid dysentery group and of *V. cholerae* filtering broth cultures and making serial passages of the organisms with the filtrates. In a duplicate series he substituted heating for filtration.

In the work to be described an attempt was made to demonstrate the presence or absence of bacteriophage in filtrates of normal stool cultures which had undergone a long period of shaking. With one exception, all of the cultures used had been in stool for several years, none less than three years. A strain of *Escherichia coli* was used which was isolated from a stool culture three weeks before being tested. The following organisms were employed in the experiments: *Aerobacter aerogenes*, *Alcaligenes fecalis*, *Bacillus fusiformis*, *Bacillus mesentericus*, *Eberthella typhi*, *Escherichia coli*, *Serratia marcescens* and *Spirillum rubrum* (a strain of *Sp. rubrum* which had lost the power of chromogenesis).

Forty eight hour agar cultures of these organisms were transferred to flasks containing 50 c.c. of peptone broth adjusted to P_H 8.0. The cultures were incubated overnight at 37° C., and then placed in a shaking machine which made approximately 230 vertical excursions per minute, over a range of one and one fourth inches.

The shaking was carried on continuously for one hundred and one hours. At the end of this time the cultures were removed, direct smears were made and agar slants inoculated. The cultures were then placed in the refrigerator for four days, and filtered through Mandler filters.

A control series was run by inoculating a second set of flasks which were kept for six weeks (four weeks at 37° C. and two weeks at room temperature) before being filtered and tested. All of the cultures used were shown by cultivation on agar to be ultrapure, growth was normal and gave no indication of the presence of a bacteriophage.

Growth occurred on the agar slants of every culture made immediately after shaking. Although this indicates that the organisms were not completely destroyed by the prolonged shaking evidence was obtained that the

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majority of the bacterial cells had been broken up. Giam-stained smears, made immediately after shaking, showed numerous disintegrated cells and considerable amorphous matter, with only a small number of intact bacteria.

The filtrates of all cultures were entirely free from bacteriophage, both the filtrates of the cultures which had been shaken, and those of the controls allowed to stand at incubator and room temperatures. Repeated passages of the filtrates on the homologous bacteria failed to demonstrate the presence of any latent bacteriophage. The filtrates were tested on several strains of the same or different species, as well as on the strain from which each was obtained. A summary of the results follows.

Filtrates of *Aerobacter aerogenes* were negative for the original strain, for three other strains of *A. aerogenes*, and for six strains of *Escherichia coli*.

Filtrates of *Alecaligenes fecalis* were negative for three strains of *A. fecalis*.

Filtrates of *Eberthella typhi* were negative for five strains of *E. typhi*, including the homologous strain.

Filtrates of *Escherichia coli* were negative for the original strain and for eight other strains of *E. coli*. The original strain was isolated three weeks before being used in the tests.

Filtrates of both *Bacillus fusiformis* and *Bacillus mesentericus* were negative for their homologous strains and for strains of *B. cereus*, *B. megatherium*, *B. globigii*, and *B. subtilis*.

Filtrates of *Spirillum rubrum* were negative for the original strain.

A number of investigators have found it impossible to demonstrate the presence of bacteriophage in apparently normal bacterial cultures, or have only occasionally isolated a bacteriophage from this source. Mallman¹ could not obtain a bacteriophage from any of over twenty cultures examined, but was able to demonstrate an antityphoid bacteriophage in the filtrate of a freshly isolated typhoid culture. Gildemeister and Herzberg² obtained negative results with a number of cultures. Flu,⁶ found a bacteriophage in two of fifty-three strains, comprising members of the typhoid-dysentery group and *V. cholerae*. One bacteriophage was active against *V. cholerae*, and the other caused lysis of the Hiss dysentery bacillus. Pondman⁵ obtained a bacteriophage from one of thirteen dysentery strains, this was the same strain from which Flu isolated a bacteriophage, using other methods.

It has been suggested by d'Herelle⁸ that the bacteriophage occasionally found in bacterial cultures is apparently present as a contamination, and the culture usually may be purified by colony isolation, the purified cultures being continued indefinitely free from bacteriophage. This was done with the cultures carrying bacteriophage isolated by Flu. Reichert⁹ found two out of eleven dysentery cultures contaminated by bacteriophage and was able to purify both cultures. Cultures of *E. coli* contaminated by bacteriophage, which were purified by colony isolation, have been isolated by d'Herelle.⁴

In general, the results of the experiments described above confirm this finding, filtrates of normal stock cultures which had undergone a prolonged period of shaking were shown to be entirely free from bacteriophage.

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1919 MADISON AVENUE

MORPHINE TOLERANCE*

III THE EFFECT OF COCAINE UPON DOGS BEFORE AND AFTER HABITUATION TO MORPHINE

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TWO normal dogs were given cocaine hydrochloride subcutaneously in the proportion of 15 mg per kilogram of body weight. In ten minutes both began to lick their chops and in twenty minutes were distinctly restless. Excitement increased and reached a maximum in thirty minutes in one and in one hundred and five minutes in the other. At this time they were throwing their heads about and snapping at imaginary objects in the air. Circus movements were marked. The pupils were dilated and the temperature increased, 36° C in one and 32° C in the other. Pulse and respiration were not particularly affected. At the end of four and one half hours there was still a little increased excitability, but two hours later six and one half hours after the drug was given they were almost normal.

Two dogs which had been rendered tolerant to morphine¹ were given the same dose of cocaine, viz 15 mg per kilogram. Both showed dilatation of the pupils and some excitement slight twitching or licking of the chops. The temperature rose 0.8° C in one and 1.1° C in the other. At the end of one hour the excitement had subsided and both were acting about as usual though the temperature of one continued to rise for another hour. Three and one half hours after the cocaine was given both appeared to be normal.

Two weeks after the withdrawal of the morphine these dogs were given the same dose of cocaine and showed a slightly more marked effect. Recovery was slower.

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A normal dog was given repeated doses of cocaine (Protocol I). The initial dose was 15 mg of cocaine hydrochloride per kilogram of body weight. This was followed in fifteen minutes by 5 mg and two more doses of 5 mg each were given at five-minute intervals. All doses were given subcutaneously. In the first fifteen minutes following the initial dose the temperature rose 0.7°C , and the pupil dilated slightly. In the next five minutes the temperature increased another 0.2°C , and dilatation of the pupil became maximal. In the next five minutes the temperature rose another 0.2°C and slight twitching developed. Following the third dose of 5 mg the temperature continued to rise, emetic movements were manifested, twitching became more marked and restlessness increased until, forty-two minutes from the beginning of the experiment, convulsions developed. The temperature was then up 1.6°C from the original. Intravenous injection of barbitol sodium and paraldehyde in 0.9 per cent sodium chloride solution, as suggested by Tatum, Atkinson and Collins,² was begun and after three minutes the convulsions ceased. Two minutes later the injection was stopped. Seventy-seven cubic centimeters of the solution had been injected into a dog weighing twelve kilograms. Dyspnea continued for thirty minutes, and the temperature rose another 0.3°C , but thirty minutes later, or two hours from the beginning of the experiment, the dog was sleeping quietly. The next day he was unsteady on his feet but otherwise normal.

PROTOCOL I

Dog 15—male, 12 kg

April 8, 1926

2 00 P.M.	Temperature 38.9°C	Pupils dilated
2 03 " "	Cocaine hydrochloride (5% solution)	15 mg per kilogram
2 08 " "	Temperature 39.1°C	
2 13 " "	Temperature 39.5°C	A little increase in size of pupils
2 19 " "	Temperature 39.6°C	Cocaine hydrochlor 5 mg per kilo
2 24 " "	Temperature 39.8°C	Maximal dilation of pupils Slight muscular twitching
	Cocaine hydrochlor 5 mg per kilo	
2 29 " "	Temperature 40.0°C	Cocaine hydrochlor 5 mg per kilo
2 33 " "	Temperature 40.0°C	Restlessness increasing Muscular twitching
2 38 " "	Temperature 40.2°C	Frequent twitching of muscles
2 40 " "	Twitching increasing	Tendency to circus movement, clockwise
2 42 " "	Temperature 40.5°C	
2 45 " "	Convulsions	
2 54 " "	Injection of barbitol sodium and paraldehyde begun	
2 57 " "	Convulsions stopped	Respiratory distress continues
2 59 " "	Injection stopped	Amount injected 77 cc
3 19 " "	Temperature 40.8°C	
3 30 " "	Quiet	Breathing dyspneic, especially on expiration
4 00 " "	Sleeping quietly	Breathing easier
4 30 " "	Sleeping	
5 00 " "	Apparently unconscious, but moving head and pawing with left fore foot	
5 30 " "	Making ineffectual attempts to rise	No movement in posterior extremities, which appear to be paralyzed

April 9, 1926

8 30 A.M. Unsteady on feet Appears to be normal otherwise

The same dog was habituated to morphine and the cocaine experiment repeated (Protocol II). Convulsions began twenty-five minutes after the

first dose of cocaine Twenty five milligrams of cocaine hydrochloride per kilogram had been administered Intravenous injection of barbitol sodium and paraldehyde was begun and the convulsions ceased when only 40 cc had been injected The rise of temperature was 17 C One hour and ten minutes after the experiment began, the dog was lying quietly and five hours from the commencement he was apparently normal The convulsions were less general and lasted a shorter time when the dog was tolerant to 150 mg per kilogram of morphine sulphate than when it was free from morphine

Protocol II

Dog 15—male, 8.75 kg

May 18 1926

10 26 A.M.	Temperature 39.0 C	Pupil 8 mm	Pulse 128	Respiration 16
10 28 " "	Cocaine hydrochloride (5% solution) 15 mg per kilogram			
10 34 " "	Temperature 39.1 C			
10 37 " "	Temperature 39.3 C			
10 39 " "	Temperature 39.3 C	Pupil 11 mm		
10 41 " "	Temperature 39.3 C	Pulse 140	Respiration 20	
10 43 " "	Temperature 39.4 C	Cocaine hydrochlor 5 mg per kilogram		
10 44 " "	Pupil 13 mm	Licking chops		
10 48 " "	Temperature 39.5 C	Pulse 152	Respiration 20	Cocaine hydrochlor 5 mg per kilogram
10 51 " "	Temperature 39.8 C	Muscular twitching		
10 52 " "	Throwing head about			
10 53 " "	Temperature 39.8 C	Convulsions		
10 55 " "	Injection of 4 cc barbitol sodium and paraldehyde			
10 56 " "	Quiet			
11 10 " "	Temperature 40.7 C	Pulse 160	Respiration 24	
11 40 " "	Lying quietly except for some twitching of jaw muscles			
12 00 M.	Lying quietly Snapping jaws			
3 00 P.M.	Walking about Gait a little stiff but nearly normal			
3 30 " "	Apparently normal			

Two weeks after morphine was withdrawn from this dog, it was given a single dose of 15 mg of cocaine hydrochloride per kilogram The result was the same as in the two mentioned earlier in this paper—a slightly greater effect than when morphine tolerant (Protocol III)

Protocol III

Dog 15—male, 10.5 kg

May 31 1926

10 18 A.M.	Temperature 38.9 C	Pupil 5 mm	Pulse 148	Respiration 24	Level No salivation
10 21 " "	Cocaine hydrochloride (5% solution) 15 mg per kilo				
10 30 " "	Salivating				
10 36 " "	Pupils dilated				
10 42 " "	Temperature 39.6 C	Pupil 12 mm	Pulse 168	Respiration 40	Licking chops
11 03 " "	Temperature 39.6 C	Pupil 12 mm	Pulse 166	Respiration 40	Rigid and trembling
11 22 " "	Temperature 40.0 C	Pupil 13 mm	Pulse 160	Respiration 40	Salivation less
11 23 " "	Vomited				
11 43 " "	Temperature 39.6 C	Pupil 13 mm	Pulse 180	Respiration 36	
12 01 P.M.	Temperature 39.8 C	Pupil 13 mm	Pulse 170	Respiration 44	
1 30 " "	Slight trembling and occasional licking of chops				
3 30 " "	Normal except pupils				

Another morphine tolerant dog was given divided doses of cocaine as described. Convulsions developed thirty minutes after the first injection. The temperature was then up 1.6°C . No barbitol sodium and paraldehyde was given. The temperature continued to rise steadily and death occurred thirteen minutes later. The temperature was then 43.2°C , a rise of 3.9°C from that prevailing at the beginning of the experiment. Two minutes later the temperature had risen another 0.5°C . The total amount of cocaine given was 30 mg of the hydrochloride per kilogram of body weight.

That morphine protects against the toxic action of cocaine is not borne out by our experiments. Sollmann² states that a general antagonism exists between morphine and small doses of cocaine, especially as regards the effects on temperature and metabolism. Large doses are synergistic. He quotes Chouppe⁴ as claiming that morphinists are relatively tolerant to cocaine. Mayer, Skillein and Sonnenschein, and Biberfeld report results with which ours are in general accord.

The results of experiments on dogs, rabbits, and cats in the use of morphine or morphine and atropine in conjunction with procaine or cocaine are regarded by Mayer⁵ as rather negative. He states that they indicate that morphine lessens the severity of the convulsions produced by large intravenous doses of procaine, but that neither morphine alone, nor with atropine, in the doses used, has any material influence on the toxicity of nearly fatal intravenous doses of procaine in the rabbit. The results with cocaine would seem to indicate that morphine alone increases the toxicity of cocaine for the cat by subcutaneous injection but that one is not justified in attributing any marked effect, either antagonistic or synergistic, to morphine and atropine, or to morphine alone, with cocaine.

Biberfeld⁶ experimented on two dogs. The first received a subcutaneous injection of morphine, 14.7 mg per kilogram, followed in twenty minutes by a subcutaneous injection of cocaine, 14.7 mg per kilogram. The dog had convulsions but lived. Nine days later the same dog was given the same dose of cocaine alone. Again convulsions occurred and the dog died within four hours. The other dog was habituated to morphine, 46.6 mg per kilogram. Ten minutes after receiving this dose of morphine it was given 14.5 mg of cocaine per kilogram. Convulsions were severe, and the dog died forty-two minutes after the injection of cocaine.

CONCLUSION

Cocaine produced convulsions that were less intense and of shorter duration in dogs habituated to the daily subcutaneous injection of morphine, 150 mg per kilogram, than in normal dogs.

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SOME OBSERVATIONS ON THE WASSERMANN AND KAHN REACTIONS*

By CLARA NIGG AND NILS PAUL LARSEN † HONOLULU T. H.

THERE have recently been a number of elaborate studies on the comparison of the Kahn and the Wassermann reactions

Thomas G. Hull¹ has reported on 26 000 Kahn tests compared with the Wassermann test and summarizes as follows: Kahn and Wassermann tests were made on 25,744 specimens with relative agreement in 97.8 per cent. Clinical histories on 200 specimens in which the Wassermann and Kahn tests disagree indicated that the Kahn test is more sensitive than the Wassermann test in treated cases. The advantages of the Kahn test are in the saving of labor, time, and cost; in the definite character of Kahn reactions in specimens in which the Wassermann is anticomplementary; in the comparative simplicity, and in reduction of technical errors.

Kahn, in a recent paper summarized as follows: Comparative Kahn and Wassermann results with 174 580 serums indicate that the Kahn test is somewhat more sensitive than the Wassermann as it was employed in this laboratory. Comparative Kahn and Wassermann results in 8,661 cases of syphilis under treatment also indicate greater sensitiveness of the Kahn test, etc.

Thompson and Ebel² still more recently report on a comparison of 10,000 Wassermann and Kahn tests run in parallel and report much the same with the weight of evidence in favor of the Kahn.

These are studies on very large groups in specialized laboratories. In each of these, however, there are always a number of bloods which are positive in the Kahn and negative in the Wassermann, and others which are negative in the Kahn and positive in the Wassermann.

In the attempt to analyze some of the causes of these variations and also to present a comparison series from a small laboratory where reactions are only set up twice a week in groups of from ten to thirty, this paper is presented. It is the small hospital where many things must be done by the same person which will test a new method more severely than where one person can be trained until he becomes very proficient in just that one manipulation.

During two different years the variation of these reactions has been followed in all routine bloods coming to the Queen's Hospital laboratory.

Two entirely different techniques were followed. During the first year merely routines were run by a hospital technician. This work was carried out by Miss Emily Mills. During the second year observations were very carefully controlled by a well-trained serologist. The one tube Kahn method was used during the first observations. This would correspond probably to the

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work done in the usual hospital laboratory. There were 1,122 Wassermann reactions checked with the Kahn. The results were as follows: 862 negative Wassermans, 872 negative Kahns, 12 doubtful Wassermans, 5 doubtful Kahns, 38 anticomplementary Wassermans, 210 positive Wassermans, 245 positive Kahns. Disagreements were summarized as follows: 6 anticomplementary Wassermans with positive Kahns, 32 anticomplementary Wassermans with negative Kahns, 6 doubtful Wassermans with positive Kahns, 13 negative Wassermans with positive Kahns, 7 positive Wassermans with negative Kahns.

During the second year 1,317 Wassermann reactions were carefully checked with the three tube Kahn method. The results were much the same except instead of 38 anticomplementary reactions there were only 6 during the second year, 7 doubtful Wassermans with positive Kahns instead of six, 22 negative Wassermans with positive Kahns, and 2 positive Wassermans with negative Kahns.

In this study, therefore, of routine examinations in an ordinary hospital laboratory, where science serves the practitioner, there seems definite evidence that no longer either a Kahn or a Wassermann should be reported separately, much stronger confirmatory evidence is available for the practitioner when each test checks the other. Where 13 doubtful Wassermans were checked by 13 positive Kahn reactions, it removed the doubt, where 35 negative Wassermans were checked with 35 positive Kahn reactions the clinical interpretation may have been strengthened, and in the 9 cases of positive Wassermans with negative Kahn reactions it possibly left a question of diagnosis to be checked by more study. The valuable point from the standpoint of a hospital laboratory is that these two reactions serve as an excellent check on one another and incidentally serve as an excellent check on the technique employed. The more every method in the laboratory can be controlled and counter controlled, of the more value will the laboratory be to medicine. Several very striking examples of this have come to our attention during this study, in one case a patient giving up his home, his job, and his environment because he felt the stigma of being classed as a syphilitic. This condemnation had been given by a 3+ Kahn reaction not checked by a Wassermann. He received two injections to which he had very severe reactions. One month later the technique employed on all doubtful cases, i.e., taking the blood on each of three successive days and running both a Kahn and a Wassermann, was employed and all six reactions were negative. Another case of a young woman who had been told a blood Wassermann was positive and that she was a syphilitic, took one injection and then lived five years in dread of a dreadful disease, avoiding doctors and even avoiding friends, at the end of that time without further treatment or symptoms, the blood was taken on each of three successive days and all tests were negative. The weight was lifted. These cases can be duplicated without doubt in every community. To avoid such calamities it should be insisted that, where doubtful reactions or weak reactions occur or when reactions occur not backed by clinical symptoms, the doctor should insist on blood on each of three successive days with check reactions.

CASE 1—(Table I) The Wassermann and Kahn studies on Patient No 1 who was in the hospital with a clinical diagnosis of luetic periostitis, shows a number of interesting variations which seem to be worthy of analysis (Case 1, Table I) In the first place his blood varied from a 4+ 4+ reaction on June 3 to a practically negative one on July 1 with treatment. The first point to be considered in evaluating such variations is the possibility of variations in technic and sensitivity of the Wassermann set up from day to day. The check on such a possibility is afforded by controls which were run simultaneously, as indicated. It will be noted (Table II) that positive control

TABLE I (CASE 1)

BLOOD DRAWN	INACTIVATION	DATE OF WASSERMANN	WASSERMANN	KAHN
6/3/27	1	6/ 3	4+ 4+	3+ 4+ 4+
6/7	1	6/ 7	(3-4)+ 2+	3-4+ 4+ 4+
	1 (on clot)	6/10	4+ 4+	4+ 4+ 4+
	3	6/10	- -	- -
6/14	1 (15) (1)	6/14	(3-4)+ (-3)+	3+ 4+ 4+
	3 (15)	6/17	- -	3+
	1 (35 min) (2)	6/14	(3-4)+ (2-3)+	3+ 4+ 4+
	3	6/17	2+ -	2+ 3+ 3-4+
	5	6/21	- -	2+ 3-4+ 4+
	7	6/28	- -	- -
	1 (1 hr) (3)	6/14	(3-4)+ (2-3)+	3+ 4+ 4+
	3	6/17	± -	1+ 2-3+ 3-4+
	5	6/21	- -	1+ 3+ 4+
	7	6/28	- -	- -
	1 (4)	6/17	4+ (3-4)+	2-3+ 3-4+ 4+
	3	6/21	4+ 1+	3-4+ 4+ 4+
	1 (on clot) (5)	6/21	4+ 4+	3-4+ 4+ 4+
	3	6/28	4+ -	2+ 2+
6/20	1	6/21	2+ -	3-4+ 4+ 4+
	3	6/28	- -	2+ 2+ 3+
6/27	1	6/28	- -	1-2+ 3-4+ 3-4+
	1	7/ 1	- -	2+ 2+ 4+
6/29 7 30 A M	1	7/ 1	(1-2)+ -	-+ 4+ 4+ before neosulph
5 P M 6/29	1	7/ 1	2+ ±	-+ 3+ 4+ after neosulph
7/1	1	7/ 1	± -	2+ 3+ 4+

TABLE II
POSITIVE CONTROLS

WASSERMANN		
6/ 3	(1)	4 4
6/ 7	(1)	4 4
	(2)	4 3-4
6/10	(1)	4 4
	(2)	4 3-4
6/14	(3)	4 4
	(2)	4 3-4
6/17	(3)	4 4
	(2)	4 3
6/21	(3)	4 4
	(2)	4 3-4
6/24	(3)	4 4
6/28	(3)	4 4
7/ 1	(3)	4 4

serum No 2 showed the same amount of fixation, namely 4+ - (3 to 4+), each time and was used throughout the greater portion of this study. The second point to be considered is the possibility of the deteriorating effect of standing on the strength of the reaction. This possibility was obviated by setting up the Wassermann either on the same day the blood was drawn or on the following day, except in one instance where the blood was two days old. Considering these checks on technique it would seem that the variations represented were indicative of daily fluctuations in vivo in the concentration of the substance responsible for the Wassermann reaction. It will be noted how much more constant the Kahn reaction remained. Another variation is that which follows repeated inactivations. It will be noted throughout that a third inactivation of this serum removed from the clot invariably diminished the strength of the Wassermann reaction. In order to ascertain whether or not this variation was due to heating per se or to the age of the serum plus heating, the following experiment was carried out. The serum obtained June 14 was divided into three portions, one heated fifteen minutes, another thirty-five minutes, and the third one hour. It will be noted that each serum, regardless of the length of the inactivating period, gave a (3 to 4+),—(2 to 3+) reaction. Thus, it would seem that the age of the serum standing free of the clot was probably the primary cause of variation in the Wassermann reaction rather than the period of heating. Curiously enough, the portion heated for thirty-five minutes on the first inactivation followed three days later with an additional fifteen minute inactivating period still gave a 2+ reaction, whereas the portion which had undergone only fifteen minutes inactivating the first time was entirely negative on the third inactivation.

Besides controlling the Wassermann set-up with sera of known positivity, there is a further check on technique afforded on the effect of repeated inactivation (or age of serum) on the specimen of June 7, where the serum after first and third inactivation was set up simultaneously on June 10. (See Table I.) There is some evidence pointing toward an intensifying effect on the Wassermann reaction due to continued contact of the clot. For instance, a portion of the serum obtained on June 7 was run the same day with a (3-4) + - 2+ reaction. Another portion, allowed to remain on the clot three days longer, was run on June 10 with a 4+4+ reaction. It will be noted that the positive control sera on these two days showed identical reactions, hence a variation could not be due to technical difference. Furthermore, portions of the serum obtained on June 14 and allowed to remain on the clot were run on June 14, June 17, June 21, the reactions growing slightly, though definitely stronger. This phenomenon has been noted on other occasions but does not always follow.

Tables III and IV show the effect of first and third inactivations on the same day, i.e., Table III includes sera which tend to grow weaker on repeated inactivations, whereas Table IV includes sera showing no change. No attempt was made in these cases to test out the relative amounts of reagent.

Case 2 shows the possible influence of bile on variations of reactions. This blood was from a patient suffering with a very severe typhoid, and no antiluetic treatment was given.

TABLE III

SUMMARY FIRST AND THIRD INACTIVATIONS RUN ON SAME DAY
FROM STRONG TO WEAKER—TOTAL 24

PATIENT	FIRST		SECOND		THIRD	
	WASS	KAHN	WASS	KAHN	WASS	KAHN
RR	3-2		3 1-2			
BR	3-2		2-3 ±			
LK	3-4 3-4 ±				1-2 ± -	
MO	3-4 3-4 ±				3-4 ± -	
MO	3-4 3-4 ±	4+ 4+ 4+			3-4 3 -	(2-3) 3 3
MO	3-4 3-4 ±				3 2 -	
OEK	3 3				± -	
FE	4 2-3	(3-4) 3 2			- -	3 3 2
HA	4 3		4 2			
KL	4 -	1 2 (2-3)			1 -	- 1 2
PM	4 4				- -	
IK	4 4	(3-4) 4 4			4 3-4	3 4 4
MF	4 4	2 3 4			1-2 2	1 (2-3) 3
JK	4 4				4 1	
KH	4 4				4 ±	
OL	± 1-2	2 4 4			- -	0 1 2
BF	2 3				- -	
LM	1 2	3 3 4			- ±	0 2 2
HY	4 3-4				3-4 3	
AS	4				4 3-4	
LH	4 4				4 3-4	
AG	2 2				1 1	
PK	4 3-4				4 ±	
MK	4 4					

TABLE IV

UNCHANGED—TOTAL 10

PATIENT	FIRST INACTIVATION WASSERMANN	THIRD INACTIVATION WASSERMANN
LG	4 4 4	4 4 4
KF	4 4 4	4 4 4
RF	4 4 4	4 4 4
KF	4 4 4	4 4 4
KM	4 4	4 4
HA	4 4	4 4
MK	4 4	4 4
HL	4 4	4 4

It will be noted from Table V that the blood taken on November 29, December 2, and December 3 and set up on the following day in each case gave 4+4+ reactions on the first inactivation and completely negative reactions when set up one or two days later after a second inactivation. These sera were all heavily bile stained. The patient was again bled on December 7 at which time the serum showed no evidence of bile but gave a 4+4+ Wassermann on each of three different portions of the serum which had remained in contact with the clot, the first portion a few hours the second portion one day, and the third portion two days (respectively). Wassermans repeated on two portions one day (and on a third portion) two days after a second inactivation were also 4+4+. A third inactivation two days later on one portion gave a negative Wassermann. This experiment suggests that the presence of the bile was responsible for the rapid change from positive to negative on the first three bloods obtained. A Wassermann repeated eight

TABLE V
EFFECT OF BILE ON THE WASSERMANN REACTION

BLOOD DRAWN	SERUM REMOVED FROM CLOT	INACT	DATE OF WASSERMANN	RESULTS	
				0.1 c c	0.05 c c
11/29	11/30	1	11/30	4+	3-4+
Bile stained	11/30	2	12/ 1	-	-
12/2	12/ 3	1	12/ 3	4+	4+
Bile stained	12/ 3	2	12/ 5	-	-
12/3	12/ 3	1	12/ 3	4+	4+
Bile stained	12/ 3	2	12/ 5	-	-
12/7					
Not bile stained	12/ 8	1	12/ 8	4+	4+
	12/ 8	2	12/10	4+	4+
	12/ 9	1	12/ 9	4+	4+
	12/ 9	2	12/10	4+	4+
	12/ 8	1	12/14	4+	4+
	12/ 8	2	12/15	4+	4+
	12/ 8	3	12/17	-	-
12/14	12/14	1	12/14	4+	4+
Not bile stain	12/14	2	12/17	4+	4+
	12/14	1	12/14	4+	4+
	12/14	2	12/17	1+	±
12/20		1	12/21	4+	4+

days after the bile-free blood was drawn was still 4+4+ after a second inactivation but gave a negative reaction on the tenth day after withdrawal and following a third inactivation. Since the last specimen showed no evidence of bile in the serum it was suggested that the biliary constituents in the first three Wassermanns were responsible for the change from 4+ to negative within twenty-four to forty-eight hours. To ascertain the possible effect of bile, the patient was again bled on December 14, at which time he had a bile free serum grossly. To one portion of the serum, clear bile (which had been obtained from a gall bladder at autopsy) was added. The Wassermann on the serum both with and without bile gave 4+4+ reactions in each case. These sera which were allowed to stand three days were inactivated a second time. The Wassermann on the sample to which bile had been added three days previously gave a 1+1± reaction, while the bile-free one was still 4+4+. This suggests very strongly that biliary constituents may transform a 4+ Wassermann into a negative reaction if allowed to stand in vitro for any length of time. The transformation takes place slowly since the reaction was not altered when the Wassermann was set up immediately after addition of bile.

Another phase of the study was the opportunity to check the reactions on the blood from lepers. It is often assumed that nonluetie leprous bloods give positive Wassermann reactions. This is probably not true. In Hasseltine's (Public Health Bulletin No. 141) study in which the Kolmer technique was used, there were 235 Wassermanns with 51 (or 21.75 per cent) positive reactions. In seventeen of these positive reactions he states, "It will be observed that the reaction is less positive in the first tube of the Kolmer test than in the other tubes. Kolmer states that this occurs occasionally and 'it may be assumed that it is due to the presence of natural antishoop hemolysin, but I have seen the phenomenon occur with hemolysin-free sera and believe that it is due to the presence of other serum constituents in this relatively

large amount of serum interfering with the fixation of complement by antigen and syphilis antibody.' In the cases reported in this paper this phenomenon has been rather frequent and has extended beyond the first tube in some cases and in one the expected reading was fully reversed. While it is possible that this result may be due to errors of technique, great care was taken to follow the exact procedure described. Kolmer states that he believes it due to other substances in the serum. The patients whose serums were tested in this series all received weekly intramuscular injections of the ethyl esters of the fatty acids of chaulmoogra oil. Some received the esters without iodine, though a majority received the esters with 1 per cent of iodine added. Whether this treatment produces some substance which would interfere with the Wassermann test has not been determined."

During the year we have done routine Wassermans on seventy five lepers at the Kaha Receiving Station in Honolulu with 30.6 per cent positive reactions in either the Wassermann Kaha or both. Of the twenty positive Wassermann reactions, ten showed the 'zoning' phenomenon in which the stronger fixation occurred with the lesser amount of serum. Native antishcep hemolysin was tested for by adding 0.5 cc of 2 per cent sheep cells and two full units of complement to 0.1 cc of each serum and incubated for one hour at 37° C. On the regular series native amboceptor was tested for in 744 sera with the following result

Enough amboceptor to produce 4+	hemolysis on 189
3+	108
2+	69
1+	91
-	284

Of the ten sera showing zoning one contained sufficient hemolysin to produce one plus hemolysis one gave two plus hemolysis three gave three plus hemolysis and only one produced complete hemolysis, while two contained no native hemolysin at all and two were not tested. Certainly it is very obvious that native hemolysin cannot be the sole cause of the zoning phenomenon noted since it occurred even in the absence of native hemolysin. Absorption of native hemolysin was carried out on seven of the sera which showed zoning and of these only two showed correction of the zoning both contained sufficient hemolysin to produce two and three plus hemolysis respectively, and were changed from 3+4+ reactions to 4+4+ reactions. The others showed identical reactions before and after absorption. The substance responsible for the zoning seems to be quite stable, since the phenomenon was manifested in several instances on the same serum even after repeated inactivations. Moreover it was demonstrated in the same patient on four different specimens taken on successive days. To check technique further many of the sera were run in duplicate on the same day and gave identical results. For example Patient No. 1 showed a \pm fixation in 0.1 cc and 2+ in 0.05 cc when set up in duplicate. These studies point conclusively to a factor other than either technique or native antishcep hemolysin. This zoning phenomenon was noted on the general routine Wassermans though in only 1.4 per cent. It occurred in 13.3 per cent of the Wassermans run on lepers which repre-

sented 50 per cent of the positives. In the general series it was also obtained repeatedly from the same patient on different days as well as on the same serum run in duplicate or on different days.

With reference to results on the Kahn reaction on lepers, Hasseltine says, "The results were somewhat disappointing in that a number of serums giving positive results with the acetone insoluble antigen and with the Kolmer antigen, failed to show a precipitate in the Kahn test." In our series of seventy-five leprosy bloods, of the twenty-three positive reactions with either the Wassermann or Kahn, there was relative agreement in twenty. Of the re-

TABLE VI

VARIATION OF THE WASSERMANN AND KAHN REACTIONS IN UNTREATED BLOODS
Total—12

PATIENT	DATE TESTED	SERUM OF	WASSERMANN	KAHN
1	3/ 4	3/ 4 3/ 8	1 2	~ 2 4
	3/ 8		1 ±	3 4 4
			- -	1 3 4
2	7/12		- -	1 2 3
	7/15		- -	- - -
3	7/ 5		- -	- 2 4
	7/15		- -	- - -
4	2/25		4 3	3 4 2
	3/25		4 4	- - -
5	7/ 5	7/ 6	(3-4) 4	4 4 4
	7/ 8		4 4	4 4 4
	7/26		4 4	4 4 4
6	4/ 8	4/14 4/21 4/22 4/22 4/26	4 4	3 3 3
	4/15		3 (3-4)	3 3 3
	4/22		4 4	3 4 4
	4/22		4 4	3 4 4
	4/26		4 4	3 (3-4) (3-4)
7	11/ 2	11/ 4 11/ 5 11/ 5 11/ 8	2 -	
	11/ 5		1 -	
	11/ 5		2 -	
	11/ 8		- -	
8	1/ 4	1/ 6 1/ 6	2 1	- 1 2
	1/ 7		(2-3) 2	- 1
	1/11		1 1	3
9	11/ 8	11/22 11/23 11/23 11/26 11/26 11/26 11/30 12/ 7 12/ 9	± -	- 3 4
	11/19		1+ - - -	- - -
	11/19		1+ - - -	- - -
	11/23		± -1+ - - -	- 2 1
	11/23		- - -	- 2 -
	11/26		(2-3) - - -	- - 1
	11/26		(2-3) - - -	- - 1
	11/26		2 - - - ±	- - 1
	11/30		- -	
	12/ 7		3 (2-3)	- - 3
	12/ 9		3 2	
10	3/ 9	3/ 3 3/ 4 3/ 6 3/ 7 3/ 9	(2-3) 3 2	
			2 3 (2-3)	
			(2-3) 3 3	
			(2-3) 3 3	2 3 (3-4)
			3 3 3	1 3 (3-4)
11	3/11		(1-2) (1-2)	- ± 1
	3/19		3 3	1 2 3
12	3/ 8	Taken on three successive days	1 (3-4)	± 2 2
	3/22		3-4 4	3 3 1
	3/22		(3-4) (3-4)	2 2 2
	3/22		(3-4) (3-4)	1 2 2

maining three, one gave a negative Wassermann with a 3+ Kahn one gave a 3+ Wassermann and a negative Kahn, while the other gave a negative Wassermann and a doubtful Kahn reaction. There is therefore, practically complete agreement in the Wassermann and Kahn reactions on the blood of lepers. Hasseltine's disagreement was undoubtedly due to lack of familiarity with the Kahn technic, which he admitted was a possibility.

The question of daily variations of the Wassermann reaction in untreated cases has received considerable discussion. Craig⁴ states that of ten soldiers whose bloods were taken on each of seven days the Wassermann reaction showed striking variations. O'Leary⁵ in 30 per cent of a series of thirty bloods showed a change of 4+ to negative. A recent observation⁶ showed that of fifty patients whose bloods were repeatedly tested eight showed variations in either the Kahn or the Wassermann. During the present study, repeats were run on a large number of bloods but the only ones showing any change are listed in Table VI. It will be noted that occasionally there is a change in both Wassermann and Kahn.

SUMMARY

a The Wassermann and Kahn reactions should always be run as a check on each other, and a check on the technic in every routine series of tests. Neither one nor the other is better; both are necessary if the laboratory is to be of greatest assistance.

b Repeated inactivations tend to change a positive sera to a negative, and this change is not due to the beating alone. This change varies with different sera.

c Sera allowed to remain in contact with the blood clot tend to show an increase in the substance that gives the Wassermann reaction.

d The above two changes do not occur in the same degree with the Kahn reaction.

e Bile in vitro if allowed to remain in contact with the serum tends to produce a negative reaction.

f "Zoning" is a phenomenon producing a weaker reaction with more serum. This phenomenon, although it may occur in any blood, occurs with much greater frequency in the blood of lepers and is not due to native anti-sheep hemolysin.

g Further evidence is brought forth to indicate that the amount of syphilitic substance varies on different days in the blood. It is, therefore, strongly urged that when there is any doubt regarding a reaction the test should be repeated on blood drawn on each of three successive days.

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A NOTE CONCERNING TRANSMISSIBLE LYSIS OF DIPHTHERIA AND DIPHTHERIA-LIKE BACILLI BY METHYL VIOLET*

By JOHN E. BLAIR, PH.D., NEW YORK CITY

BOTEZ¹ has claimed to have obtained bacteriolysis in series of diphtheria, pseudodiphtheria, dysentery, and anthrax bacilli by the addition of a loopful of a saturated alcoholic solution of methyl violet to broth cultures of these organisms. According to Botez' experiments, diphtheria and anthrax bacilli are dissolved in twenty-four hours, and dysentery bacilli after about forty-eight hours, pseudodiphtheria bacilli reduce the first three loopfuls of the dye, but are dissolved after the fourth addition of methyl violet. The lysis may be continued in series, according to Botez, by transferring 0.5 to 1.0 cc. of liquid from a dissolved culture to a freshly inoculated broth tube, or by reinoculating a dissolved culture with one loopful from a broth culture of the organism being tested.

The bacteriolysis in series reported by Botez has been questioned by Poletti² and Tomaselli³. Tomaselli, for example, found that lysis no longer occurs when, in serial transfers, the concentration of the dye becomes sufficiently low. He was also able to show that bacteriophages active against three types of dysentery bacilli and against the colon bacillus were capable of causing lysis of their respective organisms in the presence of methyl violet when the dye was so dilute as not to inhibit growth of the bacteria. With a concentration of dye strong enough to retard bacterial growth, bacteriophage action was delayed.

In the work to be described, Botez' experiments were repeated, using six strains of *C. diphtheriae*, one of *C. hofmanni*, and one of *C. aerosis*. Saturated alcoholic solutions of three brands of methyl violet were used. For every experiment, five eighteen-hour broth cultures of each organism tested were employed, three tubes received one loopful each of the respective methyl violet solutions, the fourth and fifth were kept as controls, one tube receiving one loopful of 96 per cent alcohol, and the other remaining untreated. One loopful of each of the methyl violet solutions was inoculated into a sterile broth tube.

The experiment was repeated three times. In every case the results were practically identical, and may be summarized as follows. Following the introduction of one loopful of each saturated alcoholic solution of methyl violet into young broth cultures, and incubation at 37° C. for twenty-four hours, no lysis was observed in any of the eight strains employed. There was also no apparent increase in growth as compared with the controls. Tubes to which one loopful of 96 per cent alcohol had been added showed growth as abundant as the untreated controls. When loopfuls from the tubes containing methyl

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violet were inoculated on to dextrose agar plates no growth occurred. This was due apparently to the dye carried over in the loopful of culture, since a loopful inoculated into broth, with the resulting dilution of dye, gave rise to typical growth. Loopfuls from the control tubes untreated or containing 96 per cent alcohol, gave typical growth on dextrose agar and in broth. Sediment in the tubes containing methyl violet, while deeply stained showed organisms of typical morphology in smears and gave typical growth when transferred to broth.

Botez reported that the pseudodiphtheria bacilli which he used reduced the first three additions of methyl violet but underwent lysis upon the addition of a fourth loopful. In three repetitions of Botez' experiment a strain of *C. hoffmanni* reduced one brand of methyl violet once. After the addition of a second loopful of the methyl violet solution no further reduction occurred neither was any lysis apparent.

Cultures containing one loopful of a saturated alcoholic solution of methyl violet were filtered through Mandler filters and the filtrates were inoculated from young broth cultures of the homologous organisms. After incubation overnight this culture was filtered, and the filtrate again inoculated. After only one or two serial passages the organisms grew in the filtrates as readily as in control broth tubes. Apparently there was no substance in the original tubes capable of bringing about lysis of the bacteria which could be continued in series. Lack of growth or slow growth seemed to be due to inhibition by the dye which could be removed by sufficient dilution.

As a check on the inhibitory powers of methyl violet dextrose agar plates were made containing dilutions of the dye varying from 1:500 to 1:1,000,000. One half of each plate contained untreated dextrose agar as a control, the other half contained dextrose agar to which had been added sufficient methyl violet solution to give the desired dilution. The plates were made by first pouring dextrose agar as for ordinary agar plates. After the medium had solidified half of it was removed with a sterile knife and in its place was poured dextrose agar containing the proper dilution of the dye. This gave a sharp line of demarcation between control and test halves of the plate. Plates so prepared never gave evidence of contamination during the course of the experiments. When inoculating the entire plate was heavily streaked from a young broth culture of the strain being employed and incubated at 37° C.

Eight strains of diphtheria and diphtheria like bacilli were tested in this manner against ten dilutions of methyl violet covering the range mentioned above. A 1:1000 dilution of 96 per cent alcohol was also used in one series of experiments. It was found that six of the eight cultures were not inhibited by any dilution of methyl violet above 1:20,000. This included four strains of *C. diphtheriae* and one strain each of *C. hoffmanni* and *C. xerosis*. Two strains of *C. diphtheriae* were inhibited to some extent by dilutions up to 1:50,000 and 1:100,000 respectively. In all cases growth was absent or extremely scanty when the dilution of methyl violet was 1:4000 or less. The plates containing a 1:1000 dilution of alcohol showed no inhibition of growth.

From 1:4000 to the limit of inhibition, growth became progressively more abundant, in proportion to the increasing dilution of dye, and beyond the limit of inhibition, growth was equally good on both halves of the plate. On plates where inhibition was marked, the bacterial growth on the control side of the plate never extended to the junction of the plain and the methyl violet agar, because of diffusion of the relatively concentrated dye into the control agar. The distance from the middle of the plate at which bacterial growth ceased was in direct proportion to the concentration of the dye in the medium.

SUMMARY

Three repetitions of Botez' experiment, using three brands of methyl violet, resulted in obtaining inhibition of eight strains of diphtheria and diphtheria-like bacilli, but no lysis. The bacilli in the cultures to which methyl violet was added showed typical morphology, and were capable of growth when inoculated into sufficient broth to increase the dilution of the dye. Filtrates of cultures to which methyl violet had been added contained no lytic substance, as the organisms grew abundantly after one or two serial passages. By cultivation of the bacteria on dextrose agar plates containing methyl violet, it was shown that six of the eight strains used were inhibited by dilutions of methyl violet up to 1:25,000. Two strains were inhibited by dilutions as high as 1:50,000 or 1:100,000.

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1919 MADISON AVENUE

SERUM COLORIMETRY AND OTHER EVIDENCE OF THE CHOLERETIC ACTION OF TOLYSIN (ETHYL ESTER OF PARAMETHYL-PHENYLCINCHONINIC ACID) IN MAN*

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APPROXIMATELY one year ago, we published in the form of a preliminary report¹ some observations upon the Choleretic Effect of Tolysin in Cholecystography. From a small series of cases, some of which were controlled by repeated injections (Table I) we concluded "that the time from the production of cholecystographic shadows, and for their attainment of maximum density can apparently be shortened approximately one-half by the administration of one gram of tolysin by mouth prior to the intravenous administration of the usual dose of sodium tetra-iodo-phenolphthalein."

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TABLE I
CONTROLLED CASES WITH AND WITHOUT TOLYSIN

HOSPITAL NUMBER	TIME		DEGREE OF DENSITY	
	FIRST APPEARED HOURS	MAXIMUM HOURS	MAXIMUM	TWENTY FOUR HOURS
18344 (tolysin)	4	6	+	0
18344 (control)	8	8	+	++
19459 (tolysin)	2	6	++	+
19459 (control)	4	8	+++	0

TABLE II

CHOLECTSTOGRAMS INTERPRETED AS BEING NORMAL IN LOUISVILLE CITY HOSPITAL FROM
OCTOBER, 1926 TO OCTOBER 1927 SHOWING APPEARANCE TIME AND MAXIMUM
DENSITY OF THE CHOLECTSTOGRAPHIC SHADOW

HOSPITAL NO	X RAY NO	GRAM TOLYSIN	SHADOW FIRST APPEARED	SHADOW MAXIMUM DENSITY
74276	19344	+	3 hr	4 hr
74629	19544	+	2	6
74503	19549	+	1	4
75295	19819	+	1	2
75340	18618	+	4	6
75531	10658	+	2	6?
75821	20085	+	1	4
75740	20099	+	1	4
75782	20231	+	1	8
76238	20220	+	1	4
78399	20299	+	6	8
76295	20320	+	1	4
76659	20517	+	1	4
78304	21527	+	1	6
78113	21316	+	2	0
78193	21291	+	6	8
78011	17732	+	6	8
77434	20893	+	12	6
77113	20785	+	12	6
76463	20572	+	12	6
79855	16847	+	10	0
78015	21508	+	12	2
85277	25648	+	12	8
82684	24235	+	12	6
83332	21563	+	8	8
82581	23977	+	12	6
81596	23384	+	12	4
82852	18696	+	12	6
81701	23692	+	1	?
85120	24299	+	7	8
84231	24992	+	7	8
84466	25322	+	6	8
			29 hr	7 hr

These early observations have been substantiated uniformly by subsequent results. In fact, the administration of tolysin as a preliminary to the intravenous administration of sodium tetra iodo phenolphthalein has become a routine in the Louisville City Hospital. This method has proved to effect a considerable economy of time.

The data upon which the present report is based were collected from two sources: firstly the routine cholectstograms which prove to be normal from the radiologic and clinical viewpoints, secondly estimations upon the rate

of elimination from the blood stream of phenyl-tetra-iodo-phenolphthalein-sodium (isoiiodokon) with and without the administration of tolysin as a preliminary drug

CLINICAL DATA

In the interim from October 1, 1926, to October 1, 1927, there have been thirty-two normal cholecystograms reported from the x-ray department of the Louisville City Hospital. In practically all instances, these observations have been substantiated by the clinical and operative data. The basis upon which these reports of normality have been made are those generally accepted criteria: time of appearance, density and contour of the shadow, and the behavior of the shadow following food. In all of these cases, one gram of tolysin per os was administered one hour prior to the intravenous injection of the usual dose of sodium tetra-iodo phenolphthalein (Table II).

The average time of appearance of the shadow was 2.9 hours. The shadows attained maximum density after 5.7 hours. While we have no similar series of cholecystograms taken under similar conditions without tolysin as a preliminary drug, yet from a review of the literature, it would appear probable that the time for the appearance and the attainment of maximum density of the cholecystographic shadow may be shortened appreciably by the use of the tolysin as a preliminary drug.

INFLUENCE OF TOLYSIN UPON THE DISAPPEARANCE-RATE OF A DRUG FROM THE SERUM

It has been conclusively demonstrated by Abel and Rowntree,² and others, that the halogenated compounds of phenolphthalein are excreted almost wholly by the liver. Also, Brugsch and Horstels³ have shown that ethyl ester of para-methyl-phenylethanoic acid (tolysin) has a powerful choleretic action in both man and animals.

It seemed desirable, therefore, to check further the action of this drug in relation to the cholecystogram by determining the rate of elimination of one of the halogenated phenolphthaleins from the blood stream with and without the preliminary use of tolysin. None of the earlier drugs used in cholecystography (sodium tetra-brom phenolphthalein and sodium-tetra-iodo-phenolphthalein) were suitable for such a determination inasmuch as a quantitative determination in the blood serum was not only a difficult technical procedure but one subject to great error. With the introduction by Graham, Cole, Coppler and Moore⁴ of a new compound, phenyl-tetra-iodo-phenolphthalein-sodium, it was found that accurate quantitative estimations in the blood serum could readily be made. They have shown that a simultaneous liver function test and a cholecystogram is not only possible but clinically practical.

The original technique as proposed by Graham et al.⁴ was followed, except that blood serum samples were obtained every fifteen minutes for one hour following the intravenous administration of the drug. It is perhaps needless to point out that each sample should be collected and handled with great care because hemolysis of any degree spoils the colorimetric readings.

⁴Manufactured by the Mallinckrodt Chemical Works, St. Louis, Mo.

Two groups of experiments were performed. Each group consisted of three patients (under twenty years of age) who were in good health and showed no clinical evidence of biliary disease. Each patient in each group received two injections of "iso iodokon" (phenyl tetra iodo phenolphthalein sodium) one following and the other without the oral administration of one gram of tolysin.

In the first group the tolysin experiments were performed first and five days later the same experiment was repeated without the drug (Table III). In the second group the order of events was just reversed the control experiment without tolysin being first, and the tolysin experiment five days later (Table IV).

From the analysis of the data in Tables III and IV it at once becomes apparent that the drug iso iodokon is eliminated from the blood stream at a more rapid rate when tolysin is used than when the drug is omitted. In the first three experiments, when the tolysin experiment preceded by five days the control one there was a marked retention of the drug at the second injection. In the other group when the control preceded the tolysin experiment this retention is not found in fact the drug is eliminated somewhat faster than in the control experiment.

TABLE III

FIRST EXPERIMENT WITH TOLYSIN REPEATED IN FIVE DAYS WITHOUT TOLYSIN. FIGURES REPRESENT THE PERCENTAGE OF DRUG RETAINED IN THE SERUM.

NAME	METHOD	15 MIN	30 MIN	45 MIN	1 HR.
W M R					
Hosp No 81671	1 gram tolysin	15	10	6	3
X ray No 23336	Without tolysin	30	1	12	10
M B					
Hosp No 76893	1 gram tolysin	1	12	9	
X ray No 23094	Without tolysin	30	12	Hemolyzed	
P H					
Hosp No 76569	1 gram tolysin	17	14	6	3
X ray No 22737	Without tolysin	30	15	8	

TABLE IV

FIRST EXPERIMENT WITHOUT TOLYSIN REPEATED IN FIVE DAYS WITH TOLYSIN. FIGURE REPRESENT THE PERCENTAGE OF DRUG RETAINED IN THE SERUM.

NAME	METHOD	15 MIN	30 MIN	45 MIN	1 HR.
M S F					
Hosp No 80401	Without tolysin	20	15	10	6
X ray No 22191	1 gram tolysin	15	11	6	2
B L F					
Hosp No 80187	Without tolysin	15	10	5	3
X ray No 20525	1 gram tolysin	15	10	4	3
T H					
Hosp No 76117	Without tolysin	17	8	6	3
X ray No 20386	1 gram tolysin	15	10		2

We have interpreted the facts here presented as additional evidence that tolysin possesses a well-recognizable choleretic action. From the colorimetric data, it would seem that tolysin, by its action upon the liver, hastens the elimination from the blood stream of the cholecystographic drug. This action is presumably responsible for the decrease in the time required for the production of the cholecystographic shadow.

Another interesting deduction seems indicated by the colorimetric experiments. At each repeated injection, there was a retention in the blood serum of the cholecystographic drug out of proportion to other figures obtained in a similar manner at the original injection. For instance, if one compares the percentage of retention of the controls in Table I with those in Table II, the discrepancy is readily seen. This is interpreted as being evidence of a mild toxic reaction on the liver cells caused by the first injection of the drug five days before. If present, the reaction was not, however, of sufficient magnitude to cause clinical or other laboratory signs or symptoms.

SUMMARY

1 That tolysin increases the speed of excretion of the halogenated phenolphthalein by the liver is confirmed by two sets of experiments herein presented.

First the time required for the production of and the attainment of maximum density of the cholecystographic shadow is apparently shortened by the oral administration of one gram of tolysin per os one hour prior to the intravenous injection of the usual dose of sodium-tetra-iodo-phenolphthalein.

Second the elimination from the blood stream of phenyl-tetra-iodo-phenolphthalein-sodium is hastened by the oral administration of one gram of tolysin.

2 Some evidence is presented which would indicate that phenyl-tetra-iodo-phenolphthalein in the usual dosage produces a mild toxic action upon the liver cells.

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THE STABILITY OF MERCUROCHROME SOLUTIONS*

By G. F. REDDISH, PH.D. BALTIMORE, MD

IN A PAPER on the use of mercurochrome in the treatment of gonorrhea, Von Laeum and Haer¹ made the following statement "Solutions of mercurochrome are very unstable and should not be used if they have stood longer than seventy-two hours." Since this statement is at variance with the experience of others as proved both clinically and in the laboratory, further information regarding the stability of this germicide will be of interest. The clinical data are so exhaustive that it will hardly be possible to review them here. In practice old solutions of mercurochrome have been used with results equally as good as with fresh solutions.

Swartz and Davis in their studies of the action of mercurochrome on the gonococcus found that old mercurochrome solutions were not as effective against this organism as they were when freshly prepared. On the basis of this work they recommended that only fresh solutions of mercurochrome be used in treating gonorrhea. This loss of germicidal power could not be explained, the mercury content remained the same in these old solutions, and the reaction was unchanged. Loeser, Hamburger and Konwiser² were unable to duplicate these results and proved quite the contrary to be true. They found that neither the toxicity nor the bactericidal power of 1 per cent aqueous solutions of mercurochrome was altered after standing for six months at room temperature in sealed ampules. In this case oxidation was reduced to the minimum, since the solutions were stored in sealed ampules.

Our own experience proves conclusively that aqueous mercurochrome solutions are stable when properly stored for a period of at least five years. In 1922 a 1 per cent solution of mercurochrome was made and stored at room temperature in a clean glass stoppered bottle. At the end of five years, this solution was subjected to thorough study as to its bactericidal potency and toxicity. It was found first that the concentration of mercurochrome present in the solution was reduced from the original 1 per cent to 0.949 per cent and that there was a slight precipitate of metallic mercury (this is within the limit of experimental error). This change in concentration of mercurochrome is very slight considering the length of time the solution was stored and offers another proof of the stability of the mercurochrome molecule.

The results of germicidal tests confirm the chemical analysis. It was found that the dilutions necessary to kill *L. typhosus* by the Hygienic Laboratory test were almost exactly the same as required by a fresh solution of mercurochrome. Repeated tests of the old and fresh solutions were made at the same time, and the results were the same within the limits of experi-

*From the Bacteriological Laboratory of Hyn on, Westcott and Dunning.

mental error. The Hygienic Laboratory test is well suited to laboratory control of the activity of germicides and was used for that reason. When tested by the modification of this method,⁴ the old and new solutions of mercurochrome gave the same results within the limit of experimental error. Although mercurochrome is not ordinarily used to kill *B. typhosus*, these tests prove that the bactericidal efficiency of old solutions is not reduced against this organism, and this is an index of activity which serves the present purpose, especially since this is a standardized culture and has been used for this purpose for years.

Since *Staph. aureus* is the most common suppurative pathogen met with in infections, tests of the stability of old mercurochrome solutions against this organism will be of practical importance. A standardized culture of *Staph. aureus*, which complies with the standard resistance,⁷ was used in these tests. Using the method recommended by the writer⁴ for *Staph. aureus*, it was found that with this organism also the five-year-old solution of mercurochrome was practically equal in bactericidal power with fresh solutions. When a difference of killing time was noted, it was but a matter of seconds. For example, it required fifteen seconds longer for the old mercurochrome solution to kill *Staph. aureus* than did the fresh solutions. This is of only theoretic importance, since in practice the action of mercurochrome continues very much longer than the time periods used in these tests.

When tested by a method which simulates practical conditions more closely than any proposed so far, the five-year-old mercurochrome solution gives results which duplicate those obtained with fresh solutions almost exactly. The method referred to is the Agar Cup Plate Method as proposed by Dr. L. C. Himebaugh of the Pease Laboratories (not published as yet). To perform this test a shake serum-agar (1 per cent serum in nutrient agar) culture of *Staph. aureus* is poured into a sterile Petri dish, and before it cools a glass stopper of about 1.5 to 2 cm. in diameter is placed in the center. After the agar has cooled, the glass stopper is removed and the cracks between the agar and plate sealed with melted sterile agar. Six drops of the germicide to be tested are then added to the cup and the plate incubated with an unglazed clay top. Penetration and bactericidal action is indicated by a clear zone surrounding the cup. Subculture into broth from the clear zone will prove whether the action is germicidal or bacteriostatic. The five-year-old 1 per cent solution of mercurochrome was tested simultaneously with fresh 1 per cent solution by this method in plain agar, serum agar, hydrocele agar, and bile agar, and in each case the zones obtained with the two solutions were the same. Both solutions penetrated the media in germicidal strength as follows: plain agar 1.0 cm., serum agar 0.9 cm., hydrocele agar 0.7 cm., bile agar 0.8 cm. These measurements indicate the distance from the edge of the cup to the edge of the zone around the cup showing bactericidal action. Even a 1:1000 dilution of both the old and new mercurochrome solutions gave a zone of sterilization of 0.6 cm. in plain agar and 0.4 cm. in hydrocele agar. Five per cent phenol gave a zone of 0.3 cm. in both plain and hydrocele agar. These comparative tests show that under conditions simulating as closely as possible those found in practice, old mercurochrome solu-

tions are just as effective as fresh solutions. Since practical tests are of most importance, especial emphasis must be given to the results of the agar cup plate test, and from these tests it can be concluded that old aqueous solutions of mercurochrome when properly stored in a glass stoppered bottle are just as effective under practical conditions of use as fresh solutions.

Toxicity tests* have shown that the five year old mercurochrome solution is no more and no less toxic than fresh solutions.

CONCLUSIONS

The bactericidal power and the toxicity of aqueous mercurochrome solution is not affected by holding at room temperature in a glass stoppered bottle for at least five years.

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*Toxicity tests were made by Dr David I. Macht of the Pharmacological Research Laboratories Hynson Westcott and Dunning.

LABORATORY METHODS

STUDIES IN BILIARY TRACT DISEASE*

I A COLORIMETER FOR THE MELTZER-LYON TEST

By EDWARD HOLLANDER,† B S, M D, NEW YORK

SINCE Lyon¹ introduced transduodenal biliary drainage for the diagnosis of gall tract disease, contradictory reports have appeared regarding the fundamental principles involved in the method. Many workers doubt or deny that the darker colored fraction of bile, or "B" bile, contains gall bladder bile. Instead, they maintain that the portal absorption of the magnesium sulphate that is injected stimulates the liver to discharge a concentrated secretion. This view is held despite the convincing experiments of Rous and McMaster² which proves the gall bladder epithelium is the only portion of the biliary tract that has the unique function of concentrating bile. In all the publications on the Meltzer-Lyon test the color of the bile has been described only in general terms, such as, light yellow, dark yellow, light brown, dark brown, etc., which lacks exactness for comparison. In order to limit the personal equation in the recognition and description of the colors of the bile and to obtain a concrete record of a biliary drainage, the bile should be compared with a standard color. Since I published my experiences with the Meltzer-Lyon test,³ I have made observations on the volume and color of the bile with a colorimeter, described below, in more than three thousand tests.

In order to determine the depth of color of liver bile forty cholecystectomized cases were studied. These cases had been operated upon from a few weeks to five years previous to examination. Only cases free from clinical jaundice were selected, as any obstruction, either mechanical or infectious, that is sufficient to produce clinical jaundice interferes with the free flow of bile from the biliary tract. The color of the bile was compared by transmitted daylight with various solutions in a Sahli type of colorimeter (Fig 1), using tubes with a uniform diameter (24 F). An aqueous solution of 0.7 per cent of potassium dichromate (7 grams to 1000 cc distilled water), which is light yellow in color, was selected as the standard. Bile of a darker color than this I called "B" bile. The color intensity of the "B" bile can be expressed by the volume of water that must be added to match the standard. If the volume of water is equal to that of the bile the color intensity is one, if the volume of water is twice that of the bile the color intensity is two, etc. Bile ranging in color from amber yellow to dark brown can be diluted to a good match with the standard. When the bile contains an appreciable amount of bilirubin, it takes on a green tint that only approximately matches the

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standard, but which is sufficiently accurate for clinical purposes. By plotting the volume of each specimen of bile as abscissa and the color intensity as ordinate, a curve of the bile drainage is obtained (Figs II to VIII).

In the technic I employ, the position of the duodenal tube is determined by fluoroscopic examination. This is the only exact method of knowing whether the tube is in the duodenum for not infrequently reflux bile can be obtained from the stomach. When the tube is in the duodenum 30 cc of 25 per cent magnesium sulphate is injected at body temperature and the bile is obtained by siphonage. Aspiration is done only if the bile ceases to flow, to make certain that the lumen of the tube is patent. The bile is collected in test tubes, a separate tube being used for each change in the appearance of

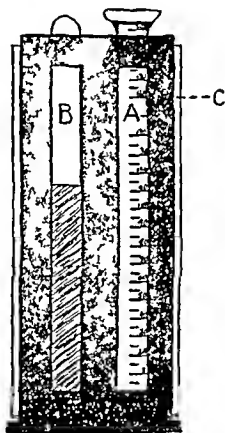
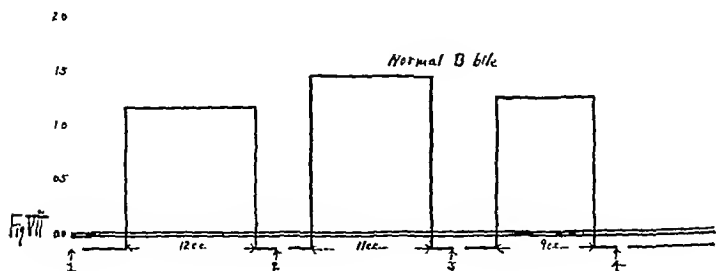
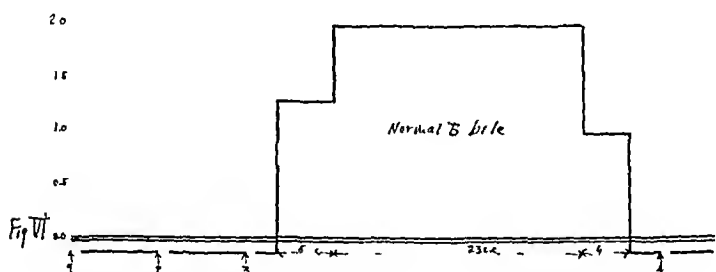
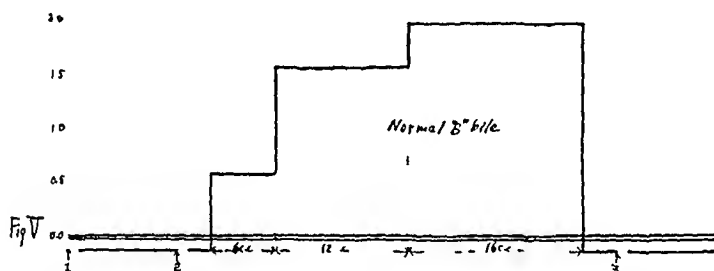
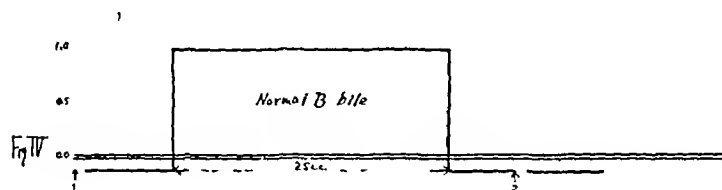
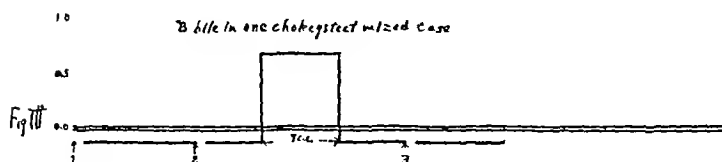
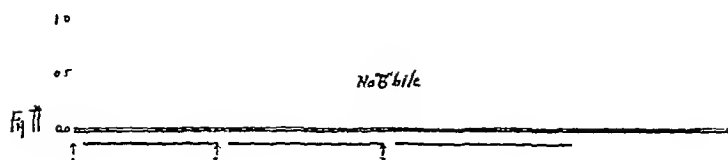


FIG 1.—The bile is placed in the graduated Tube A to about the mark 50. Less bile is used when it is very much darker than the standard and will require a large amount of dilution to match the standard. Water is added until the color of the bile matches the standard in Tube B by transmitted daylight. C is a ground glass background. The color intensity = $\frac{\text{Volume of water } (\approx \text{final reading minus volume of bile})}{\text{Volume of bile}}$

the bile. If bile drainage is obtained but no "B" bile is present, the magnesium sulphate is reinjected at ten minute intervals until three injections have been given. Occasionally the ampulla does not relax until after the second or third injection of magnesium sulphate, and the fluid obtained is colorless or only bile tinged. In such cases the three injections of magnesium sulphate are counted from the time good bile flow is first obtained (Fig II). If "B" bile is obtained, the drainage is continued until the color of the bile becomes lighter than that of the standard, when the magnesium sulphate is again injected and the collection of the bile is continued. Injections of magnesium sulphate are repeated in this manner until no more "B" bile or until the normal minimal amount is obtained (Figs III to VII). I have given as



many as six injections in one case before all the "B" bile was discharged. The examination of the bile must not be delayed for bilirubin oxidizes to biliverdin on contact with air. Hydrochloric acid causes a milky turbid precipitate with bile but since gastric juice does not usually appear in the duodenum while the "B" bile is being discharged it rarely interferes with the collection of the specimens that are necessary for examination.

In thirty eight of the forty cholecystectomized cases that were studied the color of the bile was equal to or lighter than that of the standard (Fig III). In normal subjects I have always obtained a discharge of a minimum volume of 25 c.c. of daily (or B bile) with a color intensity of one or more (Figs IV to VII). In normal cases when the "B" bile has a color intensity less than one, its volume is correspondingly increased, e.g. in one case the color intensity was only one half but the volume obtained was 60 c.c. This is equal to 30 c.c. of bile with a color intensity of one which is more than the minimal return stated for normal.

Figs II to VII

Abscissae Volume of B bile in c.c.

Ordinates color intensity of B bile

Double base line color of the standard

Horizontal lines below base line bile that is not darker than the standard (Volume not recorded)

Arrows Injections of 30 c.c. of 2 per cent magnesium sulphate

Fig II No B bile

First injection	Bile is not darker than the standard
Second injection (10 min later)	Bile is not darker than the standard
Third injection (10 min later)	Bile is not darker than the standard (10 min flow)

Fig III B bile in one cholecystectomized case

First injection	Bile is not darker than the standard
Second injection (10 min later)	7 c.c. of B bile with color intensity of 9, (Equivalent to 1/4 c.c. of bile with color intensity of one)
Third injection	Bile is not darker than the standard (10 min flow)

Fig IV Normal amount of B bile

First injection	3 c.c. of B bile with color intensity of one
Second injection	Bile is not darker than the standard (10 min flow)

Fig V Normal amount of B bile

First injection	Bile is not darker than the standard
Second injection (10 min later)	Following amount of B bile obtained 6 c.c. with color intensity of 0.6 (Equivalent to 30 c.c. with color intensity of one)
	1 c.c. with color intensity of 1.6
	10 c.c. with color intensity of .0
Third injection	Bile is not darker than the standard (10 min flow)
Total B bile is 31.6 c.c. with color intensity of 1 or more	
This is in excess of the minimal amount for normal.	

Fig VI Normal amount of B bile

First injection	Bile is not darker than the standard
Second injection (10 min later)	Bile is not darker than the standard
Third injection (10 min later)	Following amount of B bile obtained
	1 c.c. with color intensity of 1.3
	3 c.c. with color intensity of .0
	4 c.c. with color intensity of 1.0
Fourth injection	Bile is not darker than the standard (10 min flow)
Total B bile is 3 c.c. with color intensity of 1 or more	
This is in excess of the minimal amount for normal	

Fig VII Normal amount of B bile

First injection	12 c.c. of B bile with color intensity of 1.2
Second injection	11 c.c. of B bile with color intensity of 1.3
Third injection	9 c.c. of B bile with color intensity of 1.3
Fourth injection	Bile is not darker than the standard (10 min flow)
Total B bile is 32 c.c. with color intensity of 1 or more	
This is in excess of the minimal amount for normal	

METHOD

The bile salts used consisted of a mixture of 60 per cent sodium glycocholate and 40 per cent sodium taurocholate, and were prepared in a manner similar to that used by Donnelly and Mitchell. A weighed portion was dissolved in ethyl alcohol, evaporated to dryness and the residue kept over sulphuric acid. The solution was prepared by dissolving 0.1 gram in 50 cc of normal salt solution, 0.05 cc of solution, therefore, containing 0.0001 gram.

Fifteen cc of blood were drawn, 5 cc of this were used to prepare the corpuscle suspension. This was put into a test tube containing 9 cc of normal salt solution and 1 cc of 1 per cent sodium citrate. This tube was centrifuged and the corpuscles washed twice in normal salt solution. The corpuscles were then diluted with four times their volume of salt solution. The remaining 10 cc were allowed to clot and the serum withdrawn. This serum was warmed to 55° C for fifteen minutes and used at once.

In performing the test, dry 1 × 10 cc test tubes were used, and the bile solution placed in them in varying proportions. Normal salt solution was then added to make the total volume of each tube 5 cc. Two drops of the corpuscle suspension were added to each tube, and the tubes closed with div cork stoppers. They were inverted every fifteen minutes for two hours and then centrifuged. The lowest concentration of bile salts causing hemolysis was compared with a standard tube containing corpuscles and salt solution, and was considered to be the hemolytic amount.

For the series of tubes testing the inhibition of hemolysis by blood serum, 0.2 cc of serum was placed in each tube containing a similar number of corpuscles and amount of bile salt, diluted with normal salt as above. The time and procedure were then similar.

Ponder⁸ has pointed out various mistakes in technic by which a false protective power of the serum may be obtained. These are (1) the presence of hemoglobin in the serum, (2) bacterial decomposition of the serum, (3) tap

RESULTS

PATHOLOGIC		CLINICAL DIAGNOSIS	NORMAL	
CORPUSCLES	SERUM		CORPUSCLES	SERUM
0 0008	0 00615	Alcoholic cirrhosis	0 0008	0 0066
0 001	0 00615	Gallstones	0 001	0 00615
0 0008	0 0058	Cirrhosis	0 0008	0 00615
0 0008	0 0065	Gallstones	0 001	0 0066
0 0008	0 0058	Cirrhosis	0 0008	0 00615
0 0013	0 0055	Carcinoma of liver	0 0008	0 0061
0 001	0 0055	Carcinoma of liver	0 0008	0 0061
0 001	0 0058	Carcinoma of liver	0 0006	0 0061
0 0013	0 0055	Carcinoma of liver	0 0008	0 0061
0 0009	0 0058	Carcinoma of liver	0 0008	0 0061
0 0009	0 0058	Carcinoma of liver	0 001	0 0063
0 0015	0 00615	Familial jaundice	0 001	0 0065
0 0008	0 006	Familial jaundice	0 001	0 0066
0 0008	0 0051	Perniciou anemia	0 0008	0 0061
0 001	0 0058	Perniciou anemia	0 0006	0 0065
0 0008	0 0052	Perniciou anemia	0 0008	0 0061
0 0008	0 0053	Perniciou anemia	0 0008	0 00615
0 001	0 0055	Anemia, 50 per cent	0 001	0 0065
0 0013	0 0065	Anemia, 35 per cent	0 001	0 0066
0 001	0 0053	Anemia	0 0008	0 0063

water in the salt solution, and (4) exposure of the saline solution to air. As any one of these technical errors may enter into the results, it was decided to obviate them by running a normal serum as a control with each pathologic serum. The results are tabulated.

DISCUSSION

It will be observed that there is a certain definite standard for the amount of bile salts necessary to cause hemolysis of red blood cells in the normal group, as well as the protective power of the serum. This standard is slightly lower than that found by other investigators, while the amount necessary to cause hemolysis above the protective power of the serum is slightly higher. However, the corpuscles were never hemolyzed below 0.0006 gm of bile salts, or over 0.001 gm, or the serum protection below 0.0061 gm or above 0.0066 gm.

In the pathologic cases we find red blood corpuscle hemolysis never going below 0.0008 gm and in two cases going as high as 0.0013 gm. This is not a clear cut difference however, for in three cases the amount of bile salts, namely, 0.0008 gm, is the same as normal. In the protective power of the serum, we find in every case except one a definite lowering of the protective power. In the one exception that of gallstones, the two reactions were similar in both corpuscles and serum.

The two cases of familial jaundice showed wide variations. In one, the corpuscles were highly resistive (0.0015 gm), the highest recorded in the series. In the other, the corpuscles were hemolyzed below the normal control. The serum protection in both cases was high but in both cases below the protection of normal serum.

In the cases of blood disturbance the corpuscle resistance was the same as normal except in two instances. In both of these it was above normal hemolysis, one being a case of pernicious anemia, and the other an anemia of undetermined origin. In all of the cases except one, the serum protection was below that of normal. The one exception was a case of anemia of undetermined origin in which the protection was the same.

In the series of hepatic involvement caused by malignancy the red blood corpuscles were more resistant than those of the normal individual. This may be a general resistance to hemolysis and not specific for bile salts. Kondo⁹ found that injection of gall bladder bile in rabbits caused a resistance to hypertonic salt solution. The amount of bile salts necessary to cause hemolysis of red blood cells in the presence of their serum was considerably lower than that required in normal persons. In the presence of bile salts, the blood serum does not acquire added protective power. Ponder¹⁰ found similarly that repeated injections of sodium taurocholate into rabbits did not increase the inhibitory power of their serum to taurocholate hemolysis. These results are similar but not so clear cut in the case of cirrhoses and gallstones. Could it not be possible that repeated intermittent fluctuation of blood bile content, as exists in gallstones gives the serum an opportunity to return more to a normal value?

The cases of familial jaundice might be explained in the same manner.

In the clinical cases of blood disturbance, the amount of bile salts necessary to cause hemolysis of the red blood corpuscles is the same as that required in normals, with this exception in three it is higher. There is no apparent explanation for the one case of pernicious anemia. The remaining two were both in anemias of unknown origin from which both patients recovered. In these cases the entire blood system while in the process of regeneration might have the property of increased resistance in the corpuscles and increased protection in the serum. The cases of pernicious anemia showed a definite decrease in the protection of the serum below that of normal. This protection is even lower than that of hepatic involvement.

Lichtenstein¹¹ believes that, in hemolytic jaundice and pernicious anemia, disease of the bone marrow is the primary disturbance, and the irritation from the pathologic marrow acts on the blood-destroying apparatus, stimulating it to excessive function. This, of course, brings about the question of the relation of the bile pigment present in hepatic involvement to the jaundice of blood disturbance. Rich¹² was able to find bile pigment from hemoglobin in tissue cultures, but Rich and Bumstead¹³ were unable to demonstrate an enzyme having power to convert hemoglobin into bile pigment. Oppenheimer¹⁴ was unable to find any evidence that hemoglobin was transferred into bilirubin in normal persons when isolated from the general circulation or the liver.

Van den Bergh,¹⁵ in explaining his test for the presence of bile salts in the blood, contends that the direct reaction is due to a type of bile present by mechanical obstruction. The indirect is simply found in cases in which no such obstruction exists. This has been practically confirmed by later investigators.¹⁶ It has since been thought that there may be a definite relation between these two types of reactions. Retzlaff¹⁷ has confirmed the view that bilirubin in the gastrointestinal tract can be changed to the indirect van den Bergh reaction when it passes through the blood.

It is interesting to observe that in our cases of clinical jaundice, both from hepatic involvement and blood involvement, there is a distinct diminution of the protective power of the blood serum to hemolysis of the blood corpuscles by bile salts.

This raises the question of whether there could be sufficient bile salts in the blood stream caused by an obstruction of the gall bladder to cause intravascular hemolysis. In reality the actual amount of lowering of the protective power of the blood serum is relatively slight when compared to the total amount of blood serum present. Ponder¹⁸ found that the presence of 0.1 cc of blood serum lengthened the time required for hemolysis of 10 cc of a 5 per cent suspension of washed corpuscles by 1:1000 solution of sodium taurocholate from one-half to seven minutes at 37° C. Even in intense jaundice nothing like a concentration of 1:1000 is ever obtained. Hemoglobinuria is never a symptom of jaundice, therefore, bile salts must exert no hemolysis. Ponder has also shown that the bile salts enter into adsorption compounds with proteins only in the presence of acids. Therefore, as the reactions of the blood are not acid, this does not occur.

CONCLUSIONS

1 The amount of bile salts necessary to cause hemolysis of the red blood cells in the normal individual is a fairly constant value. The blood serum in the normal individual protects against a higher concentration of these bile salts and at a constant value.

2 In five cases out of six of malignancy of the liver the red blood corpuscles were more resistant to bile salts than those of a normal individual (83 per cent). In the other case (16 per cent) there was less resistance. In 5 cases, 3 of cirrhosis of the liver and 2 of gallstones, the resistance was the same in 4 and lower in one. In 6 cases of malignancy of the liver, the protective power of the serum was below that of normal and in 2 cases of gallstones and 3 cases of cirrhosis of the liver it was slightly lower than normal. In one case of gallstones it was normal.

3 In 3 of 4 cases of pernicious anemia the red blood corpuscles had the same resistance as normal; in one this was reduced. The blood serum protection was lower than that of normal serum and slightly lower than that of those from hepatic involvement.

4 In 3 cases of jaundice of undetermined origin the corpuscles were more resistant than those of the normal in 2 and the same in 1. The blood serum was lower in protective value than normal.

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A COMBINED MACROSCOPIC AND MICROSCOPIC ERYTHROCYTE FRAGILITY TECHNIC (MODIFIED METHOD OF SIMMEL)*

WITH OBSERVATIONS ON FRAGILITY IN NORMAL HUMANS

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WITH the increase in splenectomy as a therapeutic procedure, the estimation of the resistance of the erythrocytes against hypotonic saline solutions has become a more common clinical test, and its value in the differentiation of hemolytic conditions is well recognized. The history of the development of our knowledge of the subject and the elaboration of a practical technic forms a most interesting chapter in hematology, which, however, will not be taken up here.

In America today the method of Ribicire is probably most generally employed. Briefly, it consists of setting up of a series of tubes containing diminishing strengths of hypotonic sodium-chloride solution, to each of which is added one drop of blood obtained by venous puncture. It has the advantage of being read rapidly, but it has many disadvantages. These are first, the various solutions are prepared generally by a tedious drop method which is time-consuming and rather inaccurate, as variations in the size of the drops may occur unless one exercises extreme care in holding the delivery pipette at a constant angle, second, the quantity of blood employed is not constant and may, therefore, cause uncertainty in the findings, third, sodium chloride is the only salt employed, rather than a mixture of salts as they occur in the blood, and fourth, the readings are purely macroscopic and do not allow for finer microscopic readings, if such are desired.

Simmel in 1923, after a careful survey of previous methods, devised a technic which does away with many of these disadvantages. In the first place, he employs a mixture of salts as they occur in the blood serum, namely, 8.2 gm NaCl, 0.2 gm KCl, 0.2 gm $MgCl_2$, 0.2 gm $CaCl_2$, 0.1 gm NaH_2PO_4 , and 0.5 gm $NaHCO_3$ per liter of water. Such a solution has an osmotic concentration of -0.56° to -0.57° which corresponds to that of normal blood serum. Further, from this as a unit (100 per cent or 1.0) dilutions are prepared with water, representing 30, 40, 50, 60, and 70 per cent solutions, or, as he expresses it, 0.3, 0.4, 0.5, 0.6, and 0.7. Thus one obtains five fluids representing various grades of hypotonicity up to isotonic fluid. The osmotic pressure in the dilutions sinks practically proportionately with the concentration, and the hydrogen-ion concentration, because of its close approach to the neutral point, may be disregarded.

To carry out the test, six erythrocyte counting pipettes are employed. Blood is obtained from the finger or ear, and the same precautions as regards

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good flow, etc., are employed as in a regular erythrocyte count. Blood is drawn to the 0.5 mark and diluted to 101, a different one of the six fluids being employed as a diluting mixture in each of the pipettes. After thorough shaking with the ends blocked, the pipettes are allowed to stand at room temperature for at least an hour, not over two, and then after reshaking to distribute the precipitated cells a count is made of the remaining unhemolyzed erythrocytes in the usual manner by means of a blood counting chamber. One thus obtains figures representing the number of red blood cells per cubic millimeter of blood after treatment with each diluent, that of diluent 1 or 100 per cent corresponding to the full red blood cell count. These figures represent the resistance picture and, from them and comparison with the normal, one may readily detect any increased or decreased fragility. To make more vivid the changes in different diseases, etc. Simmel has introduced a method of graphic representation. This consists of a column whose height represents the total count and which is divided into blocks. The blocks are distinguished by different type of lines and show the number of cells per cubic millimeter remaining in each diluent.

The advantages of this method are obvious. First it introduces a better medium in that it corresponds to the salt composition of the blood serum. Secondly, it may be accomplished without venous puncture. Thirdly, it allows for a much more precise and accurate reading, which does not depend upon the gross evidence or lack of hemolysis. And fourthly, it opens up a whole world of finer detail in the study of resistance changes in various diseases. Not merely maximal and minimal points of hemolysis are observed, but finer variations between these points which, as Simmel has pointed out, are most interesting and of considerable diagnostic value. Bauer and Aschner, also, using a different method, have called attention to the value of such findings.

After employing Simmel's technic for some time as a routine in complete blood examinations, it occurred to us that it might be modified somewhat to advantage, and, by incorporating the principle of the Rubierre technic into it a combined macroscopic and microscopic method could be obtained which would have the good qualities of both and do away with the disadvantages. We have now been using this modified method for over two years and find it very satisfactory in every respect. Others who have become acquainted with it have likewise found it satisfactory, and it is at their suggestion that we present it in the medical literature. For the benefit of those who are not already conversant with the Simmel method the complete procedure as modified will be given in detail.

PREPARATION OF SOLUTIONS

One liter of isotonic solution consisting of a mixture of various salts as recommended by Simmel is first prepared. This is accomplished by dissolving 8.2 gm of sodium chloride (NaCl), 0.2 gm of potassium chloride (KCl), 0.2 gm of magnesium chloride (MgCl), 0.2 gm of calcium chloride (CaCl), 0.1 gm of sodium dihydrogen phosphate (NaH_2PO_4), and 0.05 gm of bicarbonate of soda (NaHCO_3), in a liter of double distilled water. The salts must

be very accurately weighed and allowance made by atomic weight for any water of crystallization. This may be referred to as the stock solution. Ten, preferably new, glass stoppered bottles are then very carefully cleaned, rinsed in distilled water, and then double distilled water, and completely dried. These are numbered respectively 100, 70, 65, 60, 55, 50, 45, 40, 35, 30 (These figures correspond to percentages of stock solution). Bottles of about 250 c.c. capacity are a handy size. In each of them, from the stock solution and double distilled water by the use of accurate volumetric pipettes, is prepared a mixture representing the percentage of stock solution conforming to the number on each bottle. This is most readily accomplished by putting 30 c.c. of stock solution in bottle "30," 35 c.c. in "35," etc., and adding sufficient water to bring each mixture to exactly 100 c.c. Bottle "100," of course, contains simply stock solution. We have departed here slightly from the technique of Summel in that not only 30, 40, 50, 60, and 70 per cent solutions

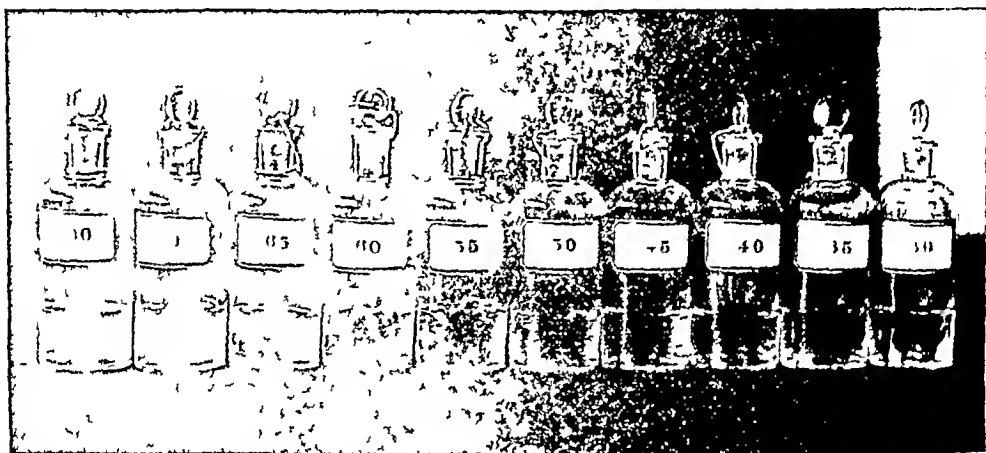


Fig. 1—Series of numbered bottles containing the various hypotonic diluents and stock solution

are employed, but also we have interpolated 35, 45, 55, 65 per cent solutions. By thus using a more gradually rising scale, the difference in hemolysis in adjacent tubes is less, finer details of change can be observed and, moreover the interpolated tube act, in a way, as a check or control on the others. This is particularly valuable in the lower solutions, where, under the original method, often great differences in hemolysis would occur. As it is upon the results of hemolysis in these solutions, especially 40 and 50 that attention is focused, and often great differences exist here, the introduction of the 45 and 55 solutions adds particularly to the value and interpretation of the test (Fig. 1).

The solutions are practically stable if left tightly corked and untouched, however, in removing fluid for the tests they are exposed to the entrance of dust, etc., and after some time may show fine sedimentation or crystallization. The bottles should then be carefully cleaned, rinsed and dried and fresh dilutions prepared from the stock.

APPARATUS AND PREPARATION FOR TEST

The following apparatus is necessary for carrying out the test: a Wassermann rack is used in the Ribierre method; ten small test tubes preferably about 10 cm long and 1 cm in diameter inside measure; a pipette to deliver one c.c., and a small pipette made to measure 5 c.mm. The last should be of sufficiently small bore to make the column of blood measured in it at least 4 cm long and not so small that discharge of contents is difficult. A very satisfactory pipette is that which comes as part of the Gower hemacytometer apparatus. Both pipettes should be carefully checked and recalibrated if necessary. The tubes must be perfectly clean and dry.

Having placed the tubes in the rack, starting from the bottle containing 30 per cent solution, 1 c.c. is pipetted from each bottle into separate tubes, carefully preserving the order. The pipette should not be rinsed in water between each bottle, but after blowing out what remains of the fluid in the tip, placed directly in the solution of the next higher bottle and rinsed in it before pipetting off 1 c.c. from it. In this way no appreciable alteration of the contents occurs and a series of 10 tubes each containing 1 c.c. of fluid, starting with the most hypotonic 30 per cent solutions and ascending to the 100 per cent isotonic solution, is rapidly set up.

CONDUCTING THE TEST

The rack with the tubes and the 5 c.mm. pipette are then taken to the bedside. Blood is obtained from the finger, in which the puncture wound should be sufficiently deep to produce a free flow. Blood is drawn to the mark in the small pipette by means of a nipple placed over the end or a piece of small tubing with mouth piece, and expelled into the first tube containing 30 per cent solution. The fluid should be drawn into the pipette once or twice to wash out all the cells. The tube is at once shaken gently until the blood is well mixed in the solution. The succeeding tube is treated in the same manner and so forth in order until all have received the blood. It is advisable to draw blood into the pipette and expel it on gauze between each tube, thus rinsing the pipette of any solution from the previous tube.

As stated above, either a rubber nipple may be placed over the end of the 5 c.mm. pipette to suck up and expel the blood or a piece of rubber tubing with a mouth piece may be used. Theoretically the former method is preferable as it does not allow carbon dioxide from the expired air, which is said to alter the fragility of the cells to play any role. The latter method however is more convenient and if one takes precautions that expired air is not blown through the solutions, is quite satisfactory and accurate for clinical work.

READING THE TEST MACROSCOPICALLY

Hemolysis of the cells takes place within a few minutes so that conclusion can be drawn almost at once. It is advisable, however, to assure completion of the process that the rack stand at room temperature for at least one half hour. At the end of that time it will be noticed that the cells which remain have partially settled allowing one to observe the supernatant fluid. The points of initial and complete hemolysis may then be readily determined at a glance. Moreover, by reshaking the tubes since exactly the same amount

of blood has been added to each, one can readily judge the approximate number of remaining unhemolyzed cells. By the turbidity and by the interrelation of these two factors, red coloration of the fluid and turbidity, one can readily make macroscopic reading and draw conclusions as regards the normal or abnormal fragility of the erythrocytes (Fig 2)

As will be shown later, there is considerable variation in fragility findings even in apparently normal individuals, however, most normal bloods give macroscopic findings as follows. Tubes 30 and 35, complete hemolysis, no turbidity, Tube 40, practically complete hemolysis with just noticeable turbidity, Tube 45, well-marked hemolysis and definite turbidity, Tube 50, definite hemolysis and well-marked turbidity, Tube 55, slight hemolysis and very marked turbidity, Tube 60, faint trace of hemolysis and apparently complete turbidity, i. e., same as higher tubes, Tubes 65, 70 and 100, no hemolysis, complete turbidity.

Even quite slight variations from the above picture, which fall within apparently normal limits, may be readily detected in cases of increased fragil-

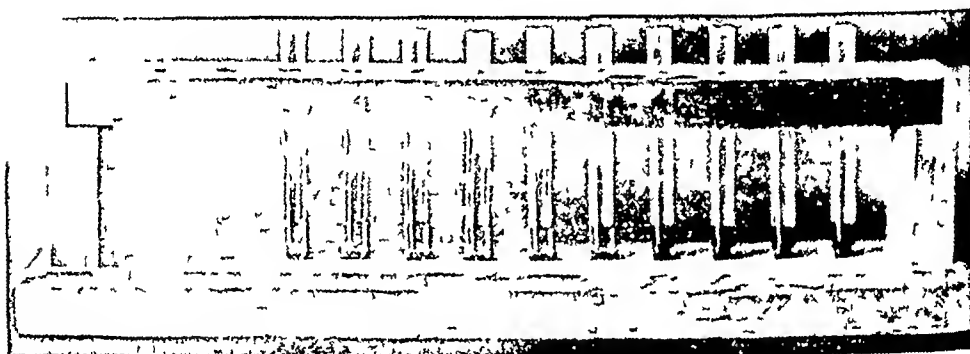


Fig 2—Rack showing completed macroscopic test. Note the turbidity in the upper and hemolysis in the lower tubes.

ity by the presence of hemolysis in the higher tubes, and in cases of increased resistance by the presence of increased turbidity in the lower tubes. In cases of severe anemia, where the number of red blood cells and hemoglobin are greatly diminished, of course, the hemolysis and turbidity factors decrease in intensity. However, the relationship remains essentially the same, and with this in mind little difficulty is experienced in drawing conclusions.

If desired, double or triple the quantity of blood may be added in very severe anemias, but this is seldom, if ever, necessary after a little experience. The method, therefore, offers macroscopically a quick and practical means of determination of the resistance of the patient's erythrocytes.

READING THE TEST MICROSCOPICALLY

Having completed the macroscopic reading, one may proceed if desired to the microscopic determination. This may appear time-consuming to many, however, with practice it is completed in a comparatively few minutes, and need not be carried out until opportunity is provided. It has several distinct advantages. First, it does away with the personal factor in judgment

of turbidity and hemolysis. Second, it allows for precise numerical expression of the extent of hemolysis in each tube, which is very valuable for the detection of finer changes of alteration in fragility, as for example in the comparison of readings in normal individuals slight day to day changes in the same patient, or in following the results of splenectomy on fragility in cases of hemolytic jaundice. Third it allows for the detection of irregular alterations as, for example, in hypochromic anemias, as chlorosis in which the lower tubes show an increased resistance of cells, while the higher show increased fragility compared with the normal. Thus a wider field of application of the test has been thrown open as pointed out by Simmel.

The microscopic determination is conducted as follows. Each tube is carefully shaken to distribute the cells evenly in the fluid. Within a couple of hours of taking the blood, this is easily accomplished but after standing a longer time it becomes increasingly more difficult due to firm sedimentation to get complete separation of the individual cells. It is advisable, therefore, if the microscopic test cannot be completed for several hours that the tubes be gently shaken from time to time during the interval. Moreover this prevents the formation of a fine web of fibrin which tends to form on standing without agitation for any considerable length of time.

One then proceeds to count by means of the regular hemocytometer the number of cells per c mm of blood remaining in each tube. The fluid is readily removed to the counting chamber by means of a capillary pipette supplied with nipple. As the dilution is 1 in 200 (more accurately 1 in 201) the computation is the same as in determining the erythrocyte count with the regular hemocytometer red cell pipette and is most easily accomplished by adding four ciphers to the total number of cells in 80 small squares. For very accurate work, the average of several enumerations should be determined.

The final result may be expressed either as cells per cubic millimeter or as percentages of the total count. Normal figures, as stated above show considerable variation, but the figures below represent a relatively common series. It will be noticed that the figures of cells per c mm are in thousands.

Tube	30	35	40	45	50	55	60	65	70	100
Per c mm	0	0	240	1 000	2 700	4 000	4 500	4 800	5 100	4 900
Per cent	0	0	4.7	19.6	52.9	78.4	88.2	94.1	100	96.1

Interesting is the fact that the highest tube, 100 very often, in fact generally, shows a slightly lower count than Tube 70. So far as we know this has never been explained.

Expression by percentages has the distinct advantage that it brings the results in all variations of total blood count to a common level. This is valuable in anemias, where otherwise we must bear in mind the relative proportion of total count to the normal in judging the figures per c mm of each tube.

Moreover, the results obtained may be graphically represented by some simple scheme as shown below in the accompanying diagrams. The column method used by Simmel does not lend itself to this modification because of the increased number of tubes, unless one substitutes various colors for the various shadings.

VARIATIONS IN NORMAL SUBJECTS

It has been recognized for some time that considerable differences in the fragility of the erythrocytes exist in apparently normal individuals. In the older methods this was expressed by variations in the points of maximum and minimum resistance. Moreover, as pointed out by Simmel, various investigators disagree as to the extremes which are considered within normal limits. More recently Leake and Pratt have published findings using the original Simmel method. They note extremely wide variations in normal subjects, and also that the erythrocytes of women are, on an average, slightly more resistant than those of men.

It seemed worth while to investigate this question using the more precise method described above, and consequently carefully conducted microscopic

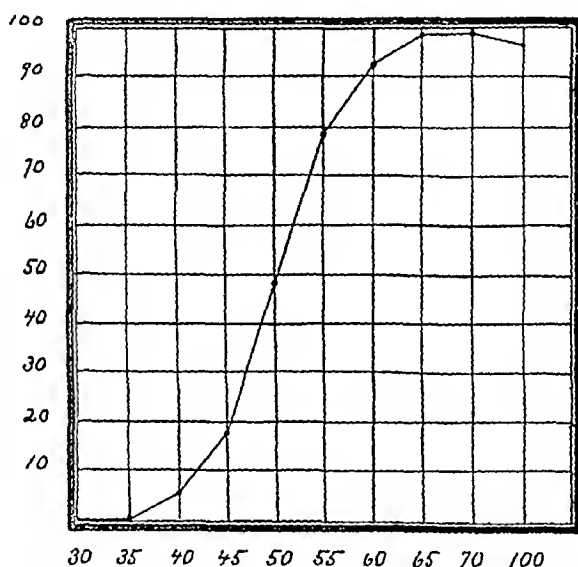


Fig 3—Hemolygram showing average of findings in fifty adult males

tests were carried out on a series of fifty adult healthy males. These consisted, with few exceptions, of medical students between twenty and twenty-five years of age. Incidentally, the other morphologic elements of the blood were enumerated and the hemoglobin estimated. Platelets were counted by the method of Spitz and the hemoglobin was computed by the Palmer method with 14 grams per 100 cc as 100 per cent. From the data obtained, which are too extensive to be given in detail here, averages were compiled and interesting observations drawn which will be briefly discussed.

An average of the fragility findings from the fifty adult males expressed in percentages of their total erythrocyte counts, together with the average of their erythrocyte, leucocyte and thrombocyte counts, and hemoglobin estimation, gave the following figures:

Tube Per Cent	30	35	40	45	50	55	60	65	70	100	RBC + 000	WBC	Plate- lets	Hb
	0	0.28	5.27	17.32	47.29	78.8	93.89	97.62	97.94	96.73	5.019	7.576	216.000	92.87

This is expressed graphically in Fig 3 of the accompanying illustrations. It will be noted, in this and the following diagrams that the numbers along the abscissae correspond to the various tubes as 30, 35, 40, etc., while those along the ordinates correspond to the percentage of unhemolyzed cells remaining in the solutions. Such a chart may for convenience be termed a 'hemolygram.' It will be noted that as one proceeds toward the higher concentrations, there are comparatively few unhemolyzed cells until Tube 45 is reached, and then a rapid rise in Tubes 50 and 55. From this hemolygram it would appear that there is always present some hemolysis in the higher tubes, but such is not the case. It is merely the result of averaging the figures.

As regards the individual variations, our findings quite agree with those of former investigators. While the majority run close to the common average about 40 per cent show considerable variation. This led us secondly to

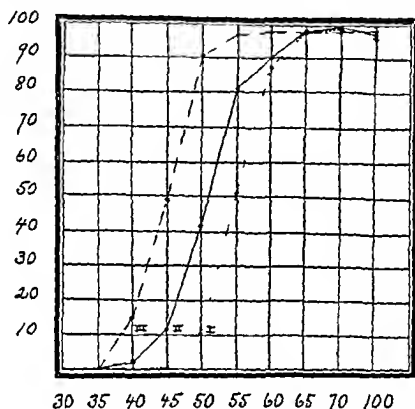


Fig 4—Hemolygram showing averages of findings with the fifty individuals separated into three groups

divide the fifty individuals into three groups: the ten showing greatest fragility (Group I), the ten showing greatest resistance (Group III) and the remaining thirty (Group II). It was necessary, because of the variation in the type of hemolysis curve, to choose some arbitrary point for this division, and as the cells on an average are about 50 per cent hemolyzed in solution 50, this point was selected as most suitable.

Averages of the findings in these three groups are as follows:

Tube	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	RNC	WBC	Platelets	Hb
Group I	0	0.1	0.6	1.84	10.77	3.93	8.7	6.97	41	98.04	96.03	4.899	7.600	00.000	9	4.00			
Group II	0	0.33	3.5	1.24	4.88	81.5	90.63	9	98	99.3	96	7.503		31	194.000	9	4.00		
Group III	0	0.37	14.36	48.4	91.09	96.53	9	6	9	8	9	08.9		08	76.0	0	000	91.9	

This is likewise graphically represented in Fig 4. It will be noted that there is in these average figures practically no difference in the general shape and character of the curves, but approximately a difference of one tube

in their positions on the graph. This would lead one to conclude that, although differences do exist in the character of individual curves, the essential difference, on an average, between the erythrocytes of normal individuals showing

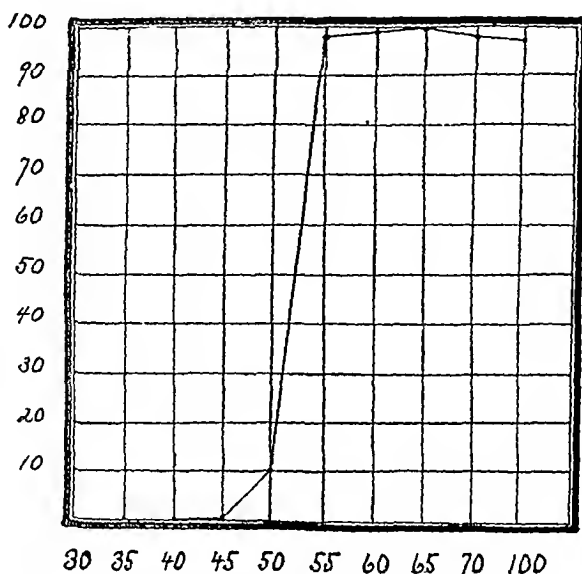


Fig 5—Hemolygram of individual showing abnormally high uniformity in the hemolysis points of his erythrocytes

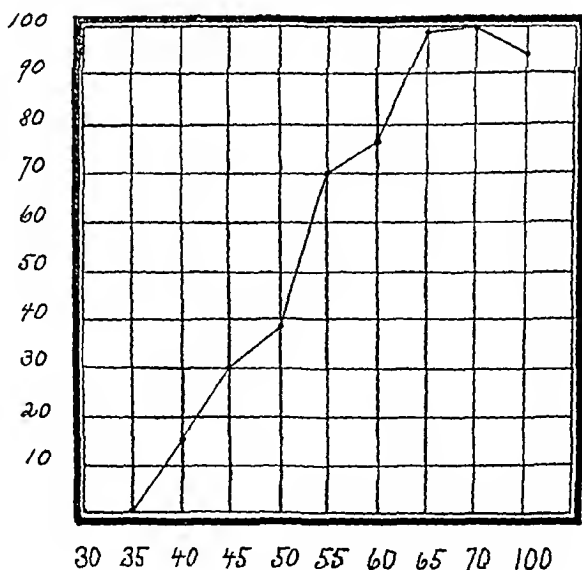


Fig 6—Hemolygram of individual showing abnormal lack of uniformity in the hemolysis points of his erythrocytes

greater or lesser hemolysis, is a general displacement of the level of hemolysis, while the relation in the number of more fragile to more resistant cells in each group remains essentially the same

Third, if one studies the character of the curve from each individual, one finds in the great majority of cases it follows that shown in Fig 4. While

a small percentage of the cells are more readily hemolyzed and a few are more resistant, from 60 to 70 per cent of them have a point of hemolysis lying within the difference expressed between three tubes, i.e., a range of 10 per cent. In the individuals with less resistant cells this range lies between Tubes 50 and 60, in those with more resistant cells between Tubes 40 and 50, while in the majority it lies between Tubes 45 and 55.

Occasional variations from this picture occur however and the findings from two such cases are shown in Figs 5 and 6. We may for instance, get the curve with very sharp rise (Fig 5) which signifies that approximately 90 per cent of the individual's cells have a point of hemolysis between two tubes, as for example here between Tube 50 and Tube 55. That is only a 5 per cent range. Such an individual has an abnormally high uniformity (isohemolysis) in the hemolysis points of his erythrocytes. On the other hand the opposite exists as shown in Fig 6. The curve here rises very gradually, signifying a relatively high percentage of more fragile and more resistant cells and an abnormal lack of uniformity (anisohemolysis).

Another interesting point is that subsequent examinations were carried out on some of the individuals several weeks after the initial examinations. The findings were essentially the same and peculiarities in the hemolysis picture appeared to be preserved. From this we are inclined to believe that individuals during health have a more or less constant and characteristic hemolysis curve, which possibly is laid down in their constitutional make up.

The study of finer abnormalities in the hemolysis picture in various disease states, as vividly expressed in the hemolygram offers a most interesting field and, there is reason to hope considerable diagnostic value.

CONCLUSIONS

1 The technique of a combined macroscopic and microscopic erythrocyte fragility test is described. This is a modification of Simmel's method. It has many distinct advantages over the usual methods now employed.

2 Normal adult males show by this method a marked individual variation in the resistance of their erythrocytes to hypotonic mixed salt solution. This variation appears to be specific and relatively constant.

3 Finer abnormalities in the hemolysis of an individual's erythrocytes are brought out by this method which opens a new field of investigation.

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IDENTIFICATION OF CULTURE MEDIA BY THE USE OF VARIOUSLY COLORED GLASS BEADS

BY FRED D WEIDMAN,* M D, PHILADELPHIA

THIS is simply a variant of the more commonly employed method of staining the cotton stoppers which has been in vogue for distinguishing between different sugar and other mediums. The disadvantages of the latter method are well known, while there is some danger of the stoppers becoming displaced or mixed up, the more important fault is that they tend to become bleached or so modified in color as to lose their distinction.

The method recommended herewith consists in placing a colored glass bead, say red, in the bottoms of the test tubes which are to receive, say, glucose medium, a blue bead in that which is to receive maltose medium, etc. If there is not a sufficient number of colored beads available, the difficulty may be overcome by using color combinations of beads or multiplying the number of beads of one color. Beads may be purchased in the Five and Ten Cent Stores very cheaply, they are perforated, but obviously this makes no difference for our purposes.

A DEVICE FOR OBTAINING UNIFORM ILLUMINATION OF COPY FOR PHOTOGRAPHIC REPRODUCTION†

BY VALENTINE SEITZ AND WM J BROWNLOW, CLEVELAND, OHIO

FOR many years, in copying charts, diagrams, and colored pictures we have used different kinds of standard studio lights and daylight. Some time ago we rigged up two perpendicular reflectors with four 200-watt lights in each, and obtained fairly satisfactory pictures. However, it was our desire to eliminate all shadows or reflections in these photographs, therefore our mechanical department endeavored to design an apparatus that would give perfect illumination and diffusion, and furnished us with the apparatus which is described below.

The apparatus consists of a reflector supported on a pipe frame 32 inches in height. The reflector, as shown in Fig 1-a, is a rectangular sheet metal housing 40 inches by 44 inches with an aperture 16 inches by 20 inches, through which the illuminated object can be photographed. The side opposite the aperture is entirely open. Twelve 200-watt lamps are distributed within this housing and are so placed that no direct light can be emitted

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through the aperture. The side in which the aperture is located faces the camera and the opposite side is directed toward the object to be photographed. By the proper arrangement of the reflecting surfaces and by the position of the lights, the indirect illumination is made absolutely uniform.

It is quite essential that the dimensions and angles indicated in the illustrations be retained and in the final set up it is necessary to block off the light emitted through the tips of the bulbs by means of opaque heat resisting paint. This is especially important when tipped lamps are used, as the lens

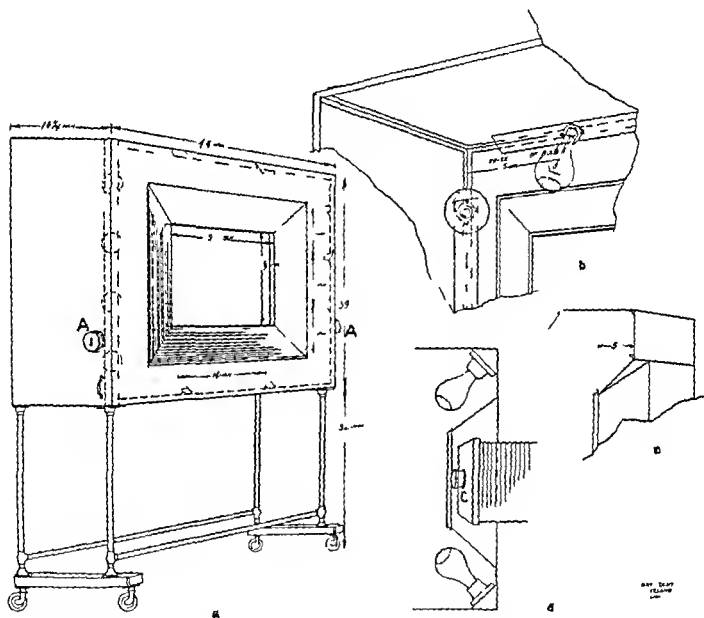


Fig. 1—Working drawing of illumination reflector for photographic copy

action of the glass at the tip produces a pattern on the illuminated object. In testing for even illumination, a flat white ground should be placed at the large opening of the reflector and by careful observation at a short distance from the apparatus, any shadows or patterns produced may be seen to move across the screen as the flat white ground is moved forward or backward.

Fig. 1 b shows the position in which the twelve 200 watt lights are placed. They are wired in two groups with the lights alternating. Switches are placed at A, A, by means of which either group can be brought into use.

In Fig. 1 c is shown a vertical section giving the angle of the walls of the aperture.

In Fig 1-*d* is shown a horizontal section giving the angle of the lights and their relation to the walls of the aperture. *C* represents a camera lens-front projected to the extreme limit within the aperture. It will be noted that no light shines into the lens at this point.

As the maximum consumption of this apparatus is 2400 watts, it is necessary to take the usual precautions regarding the size of the wire and fuses used on the illuminating line. For most copying, 1600 watts is found to be sufficient, but in copying objects, or in making "close-ups" of skin lesions, the 2400 watts permits greater speed of exposure.

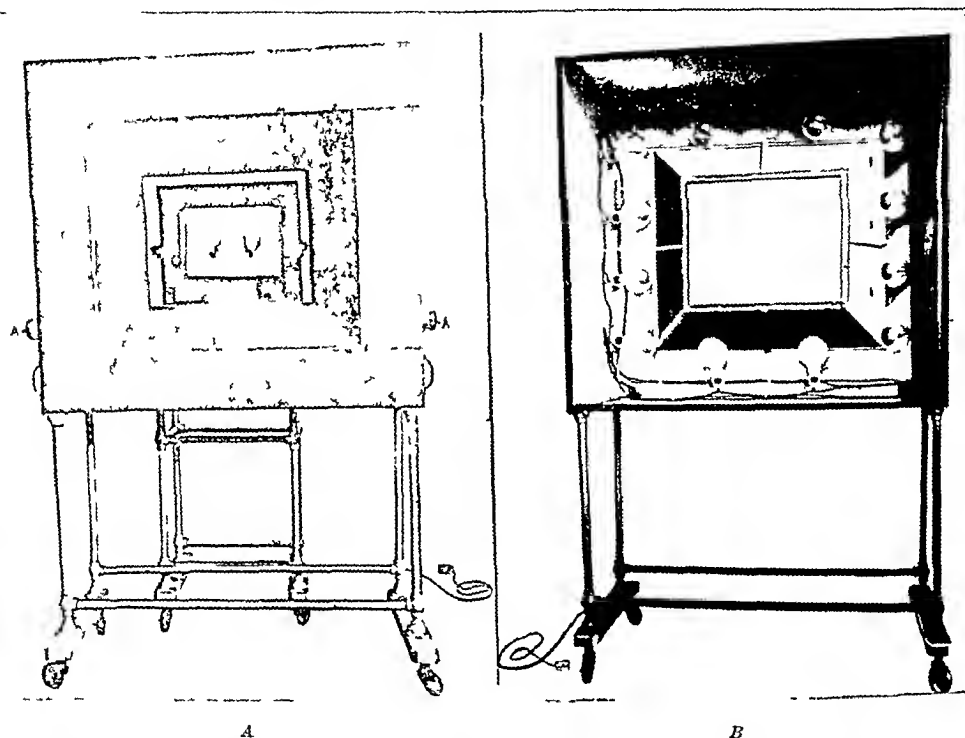


Fig. 2—Photograph of illuminating reflector for photographic copy. A, Front view showing the object to be copied in place in the press and illuminated by the reflector. B, Rear view showing the position of the illuminating units.

The photographic factors for 1600 watts are as follows

- Aperture f 32, process film, 30 to 35 seconds
- “ panchromatic film, 25 seconds (k, 3 filter)
- “ commercial film, 25 seconds
- “ Ortho film, 16 seconds

This device was designed primarily to illuminate copy held in the press described in a separate article, but it can also be used to illuminate any other object to be photographed.

AN IMPROVED PRESS FOR SUPPORTING COPY TO BE PHOTOGRAPHICALLY REPRODUCED*

BY VALENTINE SEITZ AND WM J BROWNLOW, CLEVELAND, OHIO

IN THE Art Department of the Cleveland Clinic we have been striving for a number of years to make perfect reproductions of illustrations from books and periodicals principally for lantern slides or for republication. In copying a chart or any picture with parallel lines the distortion resulting from the curve of the page at the bound edge, or from a wrinkle is especially

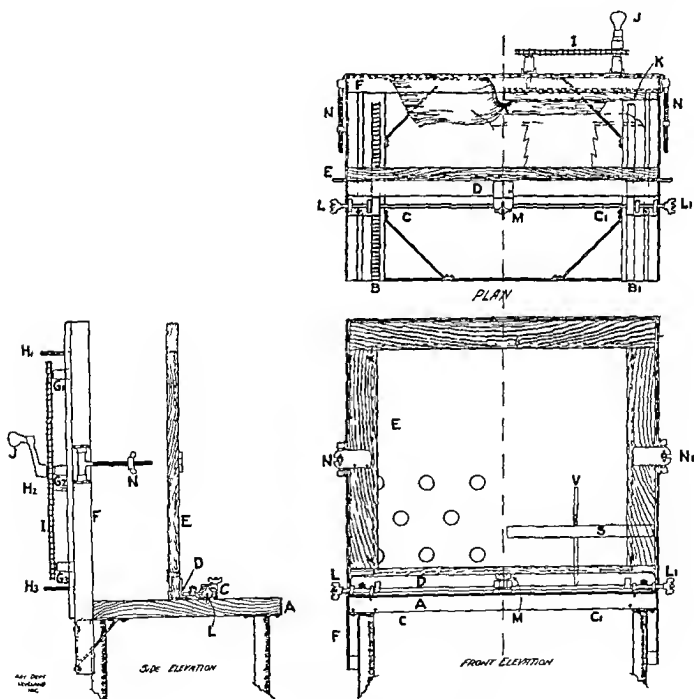


Fig 1—Working drawing of improved press for supporting copy to be photographically reproduced

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noticeable. As we were unable to find any suitable device on the market for copying book pages without injuring the book binding, we had one especially designed and built by our Mechanical Department, a description of which follows.

The press (see Fig. 1) consists of a horizontal table or bed *A*, supported by four pipe legs. On this table are mounted two racks *B*, *B'*, which engage with two pinions *C*, *C'*. The pinions are supported by a bracket *D*, on the lower end of a sliding frame *E*. This frame is of wood with an opening 15 inches by 17 inches to accommodate a $\frac{1}{2}$ inch plate glass, which may be either the full size of the frame or only half as large.

A vertical member, *F*, which is firmly connected to the bed, serves as the rear support for the book or pamphlet to be copied. This rear support is so

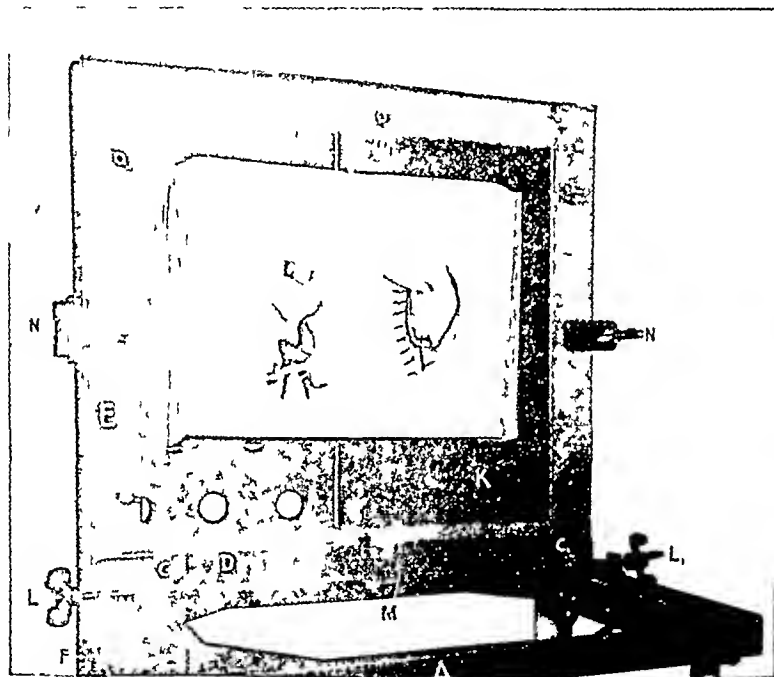


Fig. 2—Photograph of press with book in place

constructed that one side can be advanced horizontally toward the front. This is accomplished by means of three lead screws, *G'*, *G''*, *G'''*, working against a return spring action, *H'*, *H''*, *H'''*. These three lead screws are simultaneously operated by means of a chain, *I*, which engages with a sprocket on each of the screws, a crank, *J*, being directly connected to one of the sprockets.

The purpose of this mechanism is to provide for a difference of as much as one inch in the thickness of the two halves of the book to be copied. A removable wood block, *K*, provides for greater differences. Before the book is put in place, the movable half of the rear support is advanced a distance which corresponds approximately to the difference in the thickness of the two halves of the book. The book is then placed against this rear support and is held in position through the finger-holes in the stationary half of the

rear support. The glass frame is then racked toward the book by means of the handles, L, L' , with sufficient pressure to prevent the book from dropping, and the frame is locked in this position by clamping the pinion shaft at M .

Further pressure is obtained by two pivoted clamp screws and wing nuts, A, N . If two opposite pages of a book are to be photographed at the same time the full sized plate glass is used. For most work the half sized plate glass is sufficient and with this glass the insertion of the book is much easier.

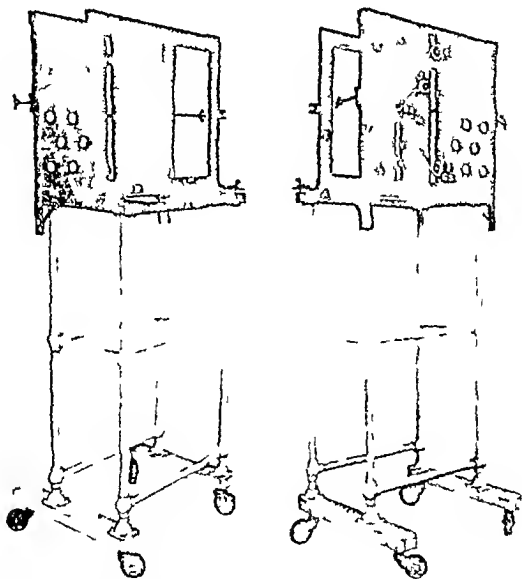


Fig. 3.—Photograph showing respectively the front and rear views of improved press

as it can be held from the front instead of through the finger holes in the stationary half of the rear support.

When a page is to be photographed which is larger than half of the frame aperture, the book can be inserted in a horizontal position with the other half extending above and over the back of the frame.

A shelf, S , can be moved up and down in a vertical slot V to accommodate various sized books and to hold them in position while the frame F is being racked into place.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M D, ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

TUBERCULIN The Specificity of the Tuberculin Reaction With Special Reference to its Histological Picture, Zieler, K Beitr z Klin d Tuberk, 1926, lxiv, 94

The author made a comparative study of intracutaneous skin inoculations with old tuberculin, triturated colon bacilli and filtrates of cultures of colon bacilli, and microphotographs of the histologic findings are presented in support of his conclusions. He finds that intracutaneous inoculations with triturated colon bacilli do produce tuberculous tissue. But this is true not only in patients with all kinds of tuberculosis but in subjects completely free of any sort of tuberculous infection. But it is not fair to compare the results of inoculation with a solution, such as that of old tuberculin, with those of inoculation of the substance of the bodies of bacteria. For the body always responds by the formation of tuberculous tissue to the administration of substances that are not absorbed quickly but are only catabolized very slowly, this is true of bacteria and triturations of their bodies, liver extracts, fats and lipoids, foreign bodies, etc. Therefore, skin reactions to old tuberculin cannot be compared with reactions to the bodies of colon bacilli but only with reactions to inoculations of filtrates of colon bacillus cultures.

Old tuberculin never produces tuberculous tissue in subjects free of tuberculosis, and inoculation with a filtrate of colon bacillus cultures does not produce tuberculous tissue either in normal individuals or ones infected with tuberculosis, but only a non-specific granulation tissue just like that produced by skin inoculations with other solutions, such as staphylococci and trichophyton or dysentery toxin. Through tuberculous tissue may be produced by different substances and is not in itself specific, yet the tuberculous tissue produced by inoculation of old tuberculin is specific and is found only in subjects infected with tuberculosis. The positive tuberculin skin reaction is a new formed tuberculous focus, for tuberculin cannot produce tuberculous tissue in an individual free of tuberculous infection.

HEMOCYTOLOGY Leukemoid Blood Pictures In Various Clinical Conditions, Krumbhaar, E B Am Jour Med Sc, October, 1926, clxvii, 4, 519

In a paper with 9 microphotographs a series of cases are reported showing that

In the 10 cases of this series blood pictures were found, which from the appearance of the blood alone would be indistinguishable from one of the various forms of leucemia. In only three, however, was the clinical diagnosis difficult.

Occasional nonspecific acute infections may respond with a lymphocytosis rather than a polynucleosis.

Acute infections may be so severe that myelocytes or even myeloblasts may be called into the blood stream in considerable numbers, especially as a terminal event.

Hemorrhage added to infection, especially in cases of neoplasm, seems to be a most potent stimulus to leucogenesis. If the stimulus is gradually increased, however, very large total counts can be reached without the appearance of noteworthy immature forms.

Inhibition or intoxication of the bone marrow may produce a leucopenia which is followed by the appearance of very immature leucocytic forms in the blood stream.

Case 7, agranulocytosis, suggests that certain bone marrow poisons may cause the appearance of very young forms in the blood stream without marked leucopenia or signs of grave disease.

Myeloma may produce a blood picture which is indistinguishable from myeloid leucemia by hematologic criteria.

Without better knowledge of the causes of the leucemias, cases associated with infection may be encountered in which definite differential diagnosis is impossible, even after an autopsy has been performed. In other words it may not be possible to distinguish between a terminal leucemoid blood picture (with bone marrow hyperplasia and infiltration of viscera) and an acute terminal true leucemia.

Case 10 shows that either leucemia in its leucemic stage may simulate Banti's disease even to the gross and histologic appearance of the spleen or that Banti's disease may be followed by leucemia or that it may terminate in an indistinguishable leucemoid picture.

DIPHTHERIA CARRIERS Shortening the Quarantine Period for Diphtheria Convalescents and Carriers Withers S Eansom J R and Humphreys E D Jour Am Med Ass October 16, 1926 1266

The authors call attention to the use of x ray for the above purpose the feasibility of the method depending on the fact that the pharyngeal lymphoid tissue is radiosensitive. A series of 54 cases are reported. All radiation was given at 50 cm target skin distance and filtered through 0.7 mm of copper and 1 mm of aluminum. In no case was more than 60 per cent of an erythema skin dose given that is just sufficient to cause some dryness of the mouth and some very slight cervical lymphadenitis in some cases.

As a rule treatments were given at the rate of 50 to 100 milliamperes minutes to each side of the head weekly. Negative cultures were obtained in an average of 9.5 days.

LABORATORY TECHNIC

BLOOD CALCIUM ESTIMATION The Estimation of Calcium in the Blood Serum, Trevan J W and Bainbridge H W Brit Biochem Jour, 1926, xx 423

A method of estimating calcium is described in which the calcium is precipitated as oxalate and converted into calcium carbonate by heating the centrifuge tube in which it is separated and washed. The calcium carbonate is titrated with acid.

The method has the advantages that the end point is more distinct than that of a permanganate titration, and all washing is carried out with saturated ammonium oxalate thus avoiding errors due to the solubility of calcium oxalate in water.

By the use of the micrometer syringe 1 cc only of serum is required and smaller quantities down to 0.1 cc will give results accurate enough for most purposes.

METHOD

Apparatus

1 Small centrifuge tubes 5 cm long with tapering bottom. The shape of the bottom of the tube is of importance the sides of the conical end must be sufficiently steep for the oxalate precipitate to slide down to the apex of the cone during centrifuging. If any oxalate adheres to the sides of the tube there is a chance of losing some during decantation of supernatant liquid. The bottom of the tube inside should be just rounded off.

2 1 cc pipettes

3 Glass stirring rods 10 cm by 15 mm made from drawn out tubing

4 Some form of microburette. The final titration for 1 cc of serum consists of the addition of about 0.2 cc of N/50 alkali. A Rehberg burette would serve, or by using 2 cc of serum as in the original Kramer and Tisdall method together with N/100 alkali for the final titration an ordinary 2 cc burette could be used. In this case, however the end point error would be rather larger.

5 Centrifuge

6 Wire holder for tube

Reagents

1 Saturated ammonium oxalate (35 per cent)

2 N/50 sodium hydroxide

3 N/100 acid We have used both phosphoric acid, as recommended by Cahen and Hurlley (1916), and hydrochloric We prefer the former The acid should be titrated against N/50 Na_2CO_3 by a special method, excess of acid should be added to the standard carbonate and the amount unneutralized titrated with the N/50 sodium hydroxide The conditions of the calcium carbonate titration are thus more closely reproduced and the titration error diminished

4 Indicator Bromphenol blue (0.04 per cent)

Methyl red (0.02 per cent) is almost as good but has to be used near the acid end of its range, and turns rather too far on the alkaline side to be entirely satisfactory for H_3PO_4 Buffer solutions, P_H 4.0 and 4.2 for bromphenol blue, 4.4 and 4.6 for methyl red, are used as comparison solutions for judging the end point The titration of the N/100 acid against standard carbonate should of course be carried out against the same indicator as that finally used for the titration of the calcium carbonate

PROCEDURE

Two cc of ammonium oxalate are measured into one of the centrifuge tubes, and 1 cc of serum is added The contents of the tube are then stirred vigorously with one of the small glass stirring rods, which is then withdrawn The amount of fluid adhering to the stirring rod may be neglected The tubes are allowed to stand for two to three hours and are centrifuged at about 3000 revolutions per minute for ten minutes The supernatant fluid is poured off and the tube drained by inversion over clean filter paper After draining for some minutes, 2 cc of ammonium oxalate are added, and the tube centrifuged again The oxalate is removed, a fresh amount added, and the centrifugation once more repeated At each centrifugation the oxalate should pack in the very apex of the conical end of the tube if the taper is correct The tubes are then dried in a steam oven to prevent spurting in the next stage The conversion into carbonate is carried out by holding the tube in a wire clip and passing it through the Bunsen flame for one minute The ammonium oxalate left is converted into ammonium carbonate which comes off as a white cloud Care should be taken to heat the whole of the tube to drive off any ammonium carbonate that may condense on the cooler parts The temperature required for complete conversion of the carbonate is comparatively low Overheating is to be avoided as the calcium oxide formed may prove very difficult to dissolve in N/100 acid The correct temperature, which is not difficult to arrive at, is indicated by the first appearance of the sodium flame around the tube The tube is cooled, 1 cc of N/100 acid added and allowed to stand for some minutes When the solution of the carbonate is complete, one drop of 0.04 per cent bromphenol blue is added from a very fine capillary pipette (the volume of the drop used is 0.016 cc) The addition of the sodium hydroxide is carried out by running a drop onto one of the small stirring rods, and then transferring it to the solution The smallest drop which can be dealt with in this way has a volume of about 0.00015 cc, which is more than sufficiently small for accurate titration This corresponds to about half a division on a metric micrometer head for the average "tuberculin syringe" The formation of drops of this size and their adequate removal by the stirring rod are much facilitated by coating the exterior of the needle of the syringe with paraffin wax The needle may be either the finest steel hypodermic needle or the glass needle described with the micrometer syringe The latter is preferable as it is easier to see that it is filled to the tip when the titration is begun Titration is carried to the point where the solution has a color intermediate between those of buffers of P_H 4.0 and 4.2 each with a concentration of bromphenol blue equal to that of the solution which is being titrated The difference between the titration figure so obtained and the titration figure for the acid alone gives the amount of calcium in the serum taken

SUMMARY

A method of estimating calcium is described in which the calcium is precipitated as oxalate and converted into calcium carbonate by heating the centrifuge tube in which it is separated and washed The calcium carbonate is titrated with acid

The method has the advantages that the end point is more distinct than that of a permanganate titration, and all washing is carried out with saturated ammonium oxalate thus avoiding errors due to the solubility of calcium oxalate in water.

By the use of the micrometer syringe 1 c.c. only of serum is required and smaller quantities down to 0.1 c.c. will give results accurate enough for most purposes.

LEPROSY Blood Chemistry Studies in Leprosy Paras E. M. Philippine Jour. Sc. June 1936 xxx 219

The sera of 100 lepers and 14 normal persons were examined and the results compared.

A study of the results collected brings out the following facts:

a. No regular correspondence can be traced between the blood findings and the duration, extent, or type of leprosy or the antileprosy treatment.

b. The average values for nonprotein nitrogen, uric acid, creatinine, and sugar for all of the leper groups were somewhat high, although many individual cases showed normal values.

c. The average values for nonprotein nitrogen and urea nitrogen are markedly high, not only in the group of lepers with ophthalmia but also in the group with lepra reactions.

d. The group averages for urea nitrogen are normal except as above stated, and those for chloride are normal throughout.

PARATYPHOID FEVER The Diagnosis of Infections with B. Paratyphosus and Allied Organisms Piney A. and Berrie A. R. Folia Hæmatologica September, 1936

The authors consider the following blood picture as characteristic of paratyphoid and allied conditions:

The total number of leucocytes is always low—it often falls below 4000 per cmm., although tending to be rather higher in young persons. It is important to remember that there may be a very transient leucocytosis at the onset of the disease.

The qualitative picture of the leucocytes also diverges widely from the normal.

The neutrophilic leucocytes show a slight increase for the first one or two days, and then there is a steady and progressive fall (in the absence of complications) until the last day of fever.

The eosinophilic leucocytes almost invariably disappear until convalescence when the ordinary postinfective eosinophilia occurs.

The monocytes (large hyaline leucocytes) seem to go through a series of quantitative changes parallel to those of the neutrophils.

The lymphocytes gradually fall during the first and second stages and then rise to numbers always relatively and often absolutely higher than normal so that they are more numerous than the neutrophils.

LEPTOSPIRA Leptospira Methods of Examination New Habitat of Free Living Forms Coles A. C. Brit. Jour. Trop. Med. June 15 1936, xxx 170

Slides must be absolutely clean!

Take two or three clean slides of about the same thickness. On one make a mark with a grease pencil towards the end. Place this slide on the stage of the microscope, which may be inclined at a comfortable angle with the 8 mm. objective and ocular No. 10—obtain a good dark background, with, of course, the tube of the microscope pulled out to the correct extent for an uncovered object (which will be about the full length of the draw tube on the Continental sized microscope). Having everything in adjustment, remove the slide. On this slide, or on another clean slide spread out a drop of the material containing leptospira in as thin a film as possible. Examine this at once under the dark ground obtained as above by a dry condenser with a stop and bellows type and the spirochetes will appear remarkably clear and sharp, much better than when examined with a coverglass and shorter tube. It is very interesting to follow the rapid movements of a

leptospira, even with the low magnification, and to notice that as soon as the film begins to dry the thread like, almost perfectly straight structure, not the least like a spirochete, begins to throw itself into coarse spirals and, immediately the film dries, the leptospira changes almost instantaneously in appearance from a delicate silvery white organism to that of a coarse, thick, yellow structure quite unlike the spirochete in the living condition. If, as soon as the film is dry, the position of the spirochete under observation be marked by making a ring around it with a diamond marker, or its exact position logged, it is quite easy to remove the slide, fix and stain the film, and examine the same organism in the stained condition, with the oil immersion objective.

Stained Preparations—*Leptospira*, like *T. pallidum*, are not easily or readily stained, but probably the best method is to fix the film while it is wet over osmic vapor, or over iodine, for a few seconds, and then dry in absolute or methyl alcohol for five to ten minutes, and subsequently staining in Giemsa in the usual way, i.e., one drop of the stain to 1 c.c. of water, overnight.

The following technic for silver staining is described.

Place the air dried or alcohol fixed thin film in a wide mouthed bottle containing acid tannic 5, acid carbolic 1, water 100. Allow it to remain three or four hours or more, the longer the time in this mordant the deeper will be the staining, but also the more the background will be stained.

Wash thoroughly under the tap, then rinse well with distilled water. Pour on the wet film a few drops of the following silver solution. To a solution of silver nitrate, 2½ or 5 per cent, in distilled water, add a few drops of concentrated ammonia till the sepia precipitate just dissolves when the solution is thoroughly shaken. Now add to this solution, very carefully, a few drops of fresh silver nitrate solution until the resulting liquid is slightly turbid, allow the silver solution to act for three or four minutes, when the preparation will turn a faint yellowish brown. Thoroughly wash the slide with distilled water. When examined, the leptospira are clearly seen.

To make the preparation a permanent one, to remove any staining of the background, and to blacken the staining of the leptospira, place the slide in ordinary toning solution that is used in photography. To about one ounce of water in a developing dish add 4 drops of a 10 per cent solution of ammonium sulphocyanide and 2 drops of a gold solution (1 gr. to 1 dr.). Allow the slide to remain in this until the film is blackened, a matter of a few minutes. Wash well with water, then pour on a 5 per cent solution of hyposulphite of soda, allow it to act for a minute or so. Finally, wash well and dry.

This method of staining or impregnating with silver has given most beautiful preparations of leptospira in water, in which the spirochetes can be picked out with quite low powers, and which show under high powers the minute spirals very distinctly. The method is absolutely fool proof, the solutions will all keep indefinitely, and the exact strength of the solutions, or the time of their action, seems of no importance.

HOOKWORM Quantitative Determination of Hookworm Ova In Feces, Hung, S. L. Arch f. Schniffs u. Tropen Hyg. 1926, xxx, 399

A measured amount of feces is emulsified in the usual way with concentrated saline. A flat shallow dish or tin vessel is filled with solid paraffin in the surface of which a depression is made of sufficient size to hold 2 gm. of feces.

The depression is filled with feces and three coverglasses floated upon the surface. These are removed after ten minutes, the number of ova counted and the number of ova in the whole amount of feces calculated from the three.

IRON IN BLOOD Micro estimation of Iron in Blood, Smirk, F. H. Biochem. Jour. London, 1927, xxi, 36

The method depends on the rapid oxidation of blood proteins by ammonium persulphate and nitric acid, with subsequent colorimetric estimation of iron as thiocyanate in the presence of acetone, against an artificial color standard. This enables a series of

estimations to be made using the same standard whereas alternatively for accurate work ing it becomes necessary to make an iron standard for each sample

Two tenths c c of blood is delivered from a standardized pipette to the bottom of a $4\frac{1}{2}$ or 5 inch Jena or Pyrex test tube. The pipette is washed by sucking two or three drops of water up and down the pipette from a small centrifuge tube, and then transferring these by the pipette to the Jena tube. The process of washing is repeated twice.

Thirty three hundredths gram of a well mixed sample of powdered ammonium persulphate is added to the contents and washed down to the bottom of the tube with 2 c c of concentrated nitric acid.

The mixture is gently heated with moderate shaking until the protein is dissolved and the solution is clear. The tube is clamped in a retort stand and heating is continued for about one minute, air being bubbled through the solution from a fine capillary tube in order to prevent bumping. The solution darkens slightly in shade and then grows lighter again. This may be taken as an index of complete oxidation though the slight color change is not always noted. The flame is withdrawn and 6 c c of distilled water are added from a burette, the stream of air ensuring mixing. The capillary tube is then withdrawn and inserted without washing into the next of the series of samples.

The first sample which has now been boiled and diluted with 6 c c of water, is poured into a 50 c c flask and the test tube washed out three times with 2 c c of distilled water from a burette each time adding the washings to the 50 c c flask. The test tube is then placed on clean blotting paper by the flask ready for the final washing with acetone.

When all the samples are dissolved and delivered into 50 c c flasks 25 c c of acetone are taken in a small measuring cylinder and the first of the series of test tubes which has already been washed with distilled water is washed out again with the acetone, the whole 25 c c of acetone washings being delivered to the first flask which is then gently shaken and immersed in cold water for five minutes. Then 5 c c of concentrated ammonium thiocyanate solution (24 gm per 100 c c of water) are added and the volume is made up to the 50 c c mark with distilled water. The iron estimation is then made colorimetrically against an artificial standard, consisting of a mixture of cochineal, methyl red, and hydrochloric acid dissolved in an equal mixture of acetone and water. The proportions are as follows:

Four c c cochineal solution (British Drug Houses Indicator)

Three and two tenths c c methyl red solution (British Drug Houses' Indicator)

Four c c dilute hydrochloric acid (1 in 5)

This is diluted with equal parts of acetone and water to the approximate depth of color required and a final adjustment of tint is made by adding drops of methyl red if the orange tint predominates and of cochineal solution if the red is too clear. The color is permanent and indistinguishable from that of the thiocyanate.

Before the ammonium thiocyanate is added to the first of a series of estimations it is best to add the acetone washings to the second of the series so that this may be cooling while the colorimetric readings are taken on the first sample.

Finally 1 c c of a solution containing 0.1 gm of ferric iron per liter (v. Fowweather) is added from an Ostwald pipette to a Jena tube. Then 0.33 gm of ammonium persulphate and 2 c c of concentrated nitric acid are also introduced and these are boiled down to about 2 c c as are the samples containing blood. The solution is made up to 50 c c as already described and compared with the artificial standard. A blood sample of equal tint to this iron sample contains 50 gm of iron per 100 c c of blood.

In explanation it is well to point out that ammonium persulphate as supplied by manufacturers contains minute traces of iron. Hence a well mixed sample must be used and the error balanced by a similar addition to the iron standard. Iron free nitric acid also gives a slight coloration with thiocyanate which is corrected by the nitric acid in the standard.

Calculation. The colorimeter is set at 15 for normal blood or 2 mm for anemic blood. The total iron minus the added iron equals the iron in gm in 0.2 c c of blood.

SYNOVIAL FLUID Comparative Studies Between Synovial Fluid and Plasma, Allison, N, Smith, F F, Dailey, M E, and Kennard, M A Jour Bone and Joint Surg, vol, 4, 758

Protein, chloride, sugar and nonprotein nitrogen have been determined in plasma and pathologic synovial fluids in twenty three instances

The protein content of the synovial fluids is less than that of the plasma The chloride content is greater than that of the plasma This inverse relationship of protein and chloride is analogous to that found between plasma and peritoneal effusions, pleural effusions, and the cerebrospinal fluid, and is probably influenced by the Donnan membrane equilibrium

Low plasma chloride is accompanied by low chloride in the synovial fluid

The nonprotein nitrogen is approximately equally distributed between plasma and synovial fluid

In fasting patients, the sugar content of noninfected synovial fluid is usually slightly lower than that of the plasma

The hyperglycemia caused by anesthesia is accompanied by a rise in sugar content of the synovial fluid

In four instances of bacterially infected fluids the sugar content was markedly lowered, while in two cases of tuberculosis of the joint, the sugar content was moderately lowered This is analogous to the low sugar content of the cerebrospinal fluid in purulent and tuberculous meningitis

It is suggested that determination of the sugar content of synovial fluids may prove to be of diagnostic value as an evidence of bacterial infection

CHOLESTEROLEMIA The Colorimetric Estimation of Cholesterol and Lecithin in Blood in Connection With Folin and Wu's System of Blood Analysis, DeToni, G M Jour Biol Chem, 1926, LV, No 1, 207

The requisite quantity of blood (5 to 10 cc) is dealbuminated by the technic of Folin and Wu The filtrate must be passed through a filter which has been thoroughly freed from all fat (The filtrate may be kept for the determination of urea, sugar, etc) The flask in which the precipitation has taken place must then be rinsed out several times with distilled water, and the water thrown onto the protein precipitate While still wet, precipitate and filter paper are placed in a small mortar and mixed carefully with 8 to 10 gm of plaster of Paris or sea sand, previously cleansed thoroughly of all fat Thus a homogeneous paste is obtained, more or less thick, according to the quantity of sea sand added

The paste is placed for one and one half to two hours in an oven at 100-105° C Afterwards triturate it carefully and transfer it to a thimble for extraction, stopping up the free aperture with cotton free of fat Use the Kumagawa Sato apparatus for the extraction and employ redistilled chloroform The extraction should take at least two hours, care must be taken to see that the siphon is in perfect working order, otherwise prolong the period of extraction When the latter is finished, the surplus chloroform must be distilled until only a residue of 10 to 15 cc remains This is transferred to a 50 or 100 cc volumetric flask, according to the amount (5 or 10 cc) of blood used for the precipitation The extraction flask is then washed out with 10 cc of chloroform three separate times, the washings being transferred to a volumetric flask The solution in the flask is finally brought up to volume

DETERMINATION OF CHOLESTEROL

Exactly 10 cc of this chloroform extract are measured out into a small flask, 4 cc of acetic anhydride and 0.2 cc of concentrated sulphuric acid are added This is then put into a dark place for half an hour at a temperature of from 20 to 30° C The color is compared with that of a standard solution composed as follows 0.15 gm of purest cholesterol (Merck) is dissolved in 100 cc of redistilled chloroform and put into small bottles containing about 15 cc each These are closed with glass stoppers, sealed with

paraffin, and kept in an ice box. By taking 10 cc of this original solution and bringing up to 100 cc with redistilled chloroform the standard solution is obtained. 10 cc of the latter contain, therefore, exactly 15 mg of cholesterol.

The calculation is simple since 10 cc of chloroform extract equal 1 cc of total blood.

DETERMINATION OF LECITHIN

The amount of lecithin in another portion of this chloroform extract can be determined by employing one of the micromethods recommended for this purpose. Whitehorn's phosphorus method, taking 5 cc of the extract is preferred.

BLOOD AGGLUTINATION *The Influence of Various Factors Upon the Hemagglutination of Red Blood Corpuscles* Higgins C C. Am Jour Med Sc October 1926 cxxviii No 4, 510

The following factors were investigated:

1 Saturation with various gases, some increasing and others delaying the rapidity with which clumping occurred.

2 Temperature. High temperatures tend to delay, low temperatures increase the rapidity of agglutination.

3 Dilution. The degree of agglutination was in inverse proportion to the degree of dilution.

4 Desfibrination. Has a negligible effect.

The author concludes that hemagglutination *in vitro* is influenced by various factors. Change in temperature has perhaps the most marked effect.

In the grouping of blood samples the temperature of the room should not be excessively elevated as clumping is delayed.

Various gases have a definite effect on hemagglutination, either prolonging it or tending to hasten the process.

POLIOMYELITIS *Further Studies of the Poliovirus Precipitin Reaction* Rosenow E C. Jour Infect Dis June 1926 xxxviii No 6 532

The results of the precipitin reaction with immune horse serum and extracts of nasopharyngeal swabbings in community and institutional outbreaks of poliomyelitis proved positive in nearly all frank and abortive cases at the time of the attack, in a high percentage of normal contacts and in persons not exposed to the disease at the time when cases occurred. It proved negative in nearly all of the cases in from two to three weeks after the acute attack had subsided and in normal persons soon after the epidemic had disappeared. In one epidemic the incidence of positive reactions generally was found low shortly before the occurrence of the first case, high during the period of the epidemic and again low after the epidemic had subsided. The increase in positive reactions as cases of poliomyelitis developed and the decrease as poliomyelitis disappeared occurred rapidly and seemingly independently of exposure to the disease, in isolated households in the country as well as in the urban population. Persons who were negative to the precipitin reaction on entrance into the epidemic zone soon became positive and reactions resembling abortive attacks of poliomyelitis were common in children. The number of positive reactions in persons who came to the Mayo Clinic from widely separated communities was relatively high during the latter part of August when poliomyelitis was generally prevalent and much lower during the latter part of October after poliomyelitis had largely disappeared. After the epidemic in Rochester had subsided and the precipitin reaction in the population had become largely negative, cases occurred south of Rochester where the number of positive precipitin reactions was high.

In certain instances poliomyelitis occurred without exposure within from five to twelve days after the presence of the streptococcus was demonstrated in the throat. Repeated swabbings showed that the carrier state lasts usually from one to three weeks in normal persons. Immunity to poliomyelitis and the occurrence of the organism in the

throat did not run parallel. The positive reactions during epidemics in adults, who are relatively immune, and in children, who are relatively susceptible, were found nearly equally high, and persons who had had poliomyelitis became carriers of the streptococcus during epidemics quite like persons who had not had the disease.

FLAGELLA STAIN A Modification of the Casares Gil Flagella Stain, Thatcher, L. M. Stain Technology, October, 1926, 1, No. 4, 143

The Casares Gil technic for staining flagella is, perhaps, better known in recent literature as the Plimmer and Paine method.

As given in Paine's paper, the stain is as follows:

1 Mordant

Tannic acid, 10 gm
Aluminum chloride (hydrated), 18 gm
Zinc chloride, 10 gm
Rosanilin hydrochloride, 15 gm
Alcohol 60 per cent, 40 cc

The solids are dissolved in the alcohol by trituration in a mortar, adding 10 cc of the alcohol first, and then the rest slowly. This alcoholic solution may be kept several years. For use, dilute with four parts of water, filter off precipitate and collect filtrate on the slide, allowing it to act for sixty seconds.

2 Stain. Carbol fuchsin

Quite by accident the writer discovered that preparations far superior to those made by the above technic were obtained if, in treating the films, a one to one water dilution of the mordant was used instead of one part to four of water as called for in the original formula. The only drawback to this modification is that filtration is more difficult, but the excellent quality of the preparations obtained in this manner would seem to justify its use.

MERCUROCHROME The Present Status of Mercurochrome 220 Soluble, Davis, H. H. Am Jour Med Sc, September, 1926, clxxv, 340

There is experimental evidence of the value of mercurochrome 220 soluble intravenously in the treatment of septicemia and other infections. Other equally convincing experimental results point to the fact that it is not bactericidal in blood, and that its use is not unattended by danger. Many clinical reports show miraculous cures, others have no benefit, and in some it has probably hastened death. Therefore, treatment with merurochrome must still be considered in the experimental stage. Because of its dangers it should not be used indiscriminately and should be reserved for desperate cases.

Mercurochrome is dangerous intraperitoneally because of the local irritant action and because of the often very severe general reaction.

If used in wounds, sinuses, or serous cavities, the dose should be limited to 5 mg per kilogram of body weight, as it is easily absorbed, and if too much is used, it may lead to severe reaction or stomatitis.

The alcohol acetone aqueous solution of mercurochrome recommended by Scott and Hill is a very satisfactory preoperative skin antiseptic. However, it should not be injected into the nose, urinary bladder, vagina, and so forth, along with a local anesthetic, as it will give a precipitate.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building,
Richmond Va

*The Duodenal Tube**

DR EINHORN reviews his contributions to the development of the duodenal tube and to its diagnostic and therapeutic use. This work began with the construction of the duodenal bucket. Among the more recent of his instruments may be mentioned the pyloric dilator and the double balloon pyloric dilator. The normal pylorus admits a balloon with a circumference of from 52 to 54 millimeters. In relative stenosis balloons larger than 44 millimeters encounter resistance. This instrument is then of value in recognizing stenosis of the pylorus. If the balloon is inflated still further the pylorus can usually be pulled along with the balloon upward almost to the cardia without much force and without pain to the patient. This when it occurs would indicate that the pylorus is free from adhesions to the gall bladder or liver. On the other hand distinct pain following traction on the distended pyloric balloon suggests adhesions around the pylorus or duodenum.

The same instrument is of use in the treatment of cardiospasm often passing into the stomach when esophageal bougie or stomach tube has failed to do so.

Dr Einhorn recommends stretching of the pylorus with his pyloric dilator in cases of pylorospasm not accompanied by fresh ulcer in healed medical ulcer in beginning benign stenosis of the pylorus and in advanced benign stricture of the pylorus when for any reason operation is contraindicated.

The jointed intestinal tube which may be allowed to pass to any length makes it possible to treat severe cases of ulcerative colitis by irrigation from above, thus avoiding colostomy or cecostomy.

The author also describes an instrument for obtaining stomach and duodenal contents simultaneously through the same tube. He has perfected the gastric introducer, an applicator which may be loosely attached to the olive of a duodenal tube and used to push the tube into the stomach when the patient finds himself unable to swallow the tube. The applicator is then removed leaving the tube in place.

The intestinal delineator is a long wire thread which will demonstrate with the aid of fluoroscopy the entire course of the intestinal tract.

At the end of the delineator is a small metal ball. Normally this passes ahead of the thread at all times so that in the small intestine the thread stretches out as one continuous line without curls or kinks. The author believes that in intestinal obstruction the stoppage of the ball with consequent tangling of the thread and the advance of the thread beyond the ball at this point would be of diagnostic import. He finds its chief usefulness in the diagnosis and study of cardiospasm and pylorospasm. Here the very fine copper thread delineates the contour of the lumens of these two valves more clearly than does the opaque meal. He believes that earlier stages of spasticity of the pylorus can be recognized than with the contrast meal. Furthermore study of the valves can be carried on over a longer period and intermittent spasticity may be thus demonstrated.

The technique and results of duodenal illumination as developed by Einhorn are presented in detail.

*The Duodenal Tube and Its Possibilities By Max Einhorn M.D. Professor of Medicine of New York Postgraduate Medical School Visiting Physician to the Lenox Hill Hospital New York Cloth Illustrated Pp 706 F. A. Davis Company Philadelphia

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*The Bacteriophage and Its Behavior*¹

AFTER the living cell had been recognized as the smallest form of minute matter, the question gradually obtruded itself, can life exist in any conceivable state smaller than the cell? Knowledge of the existence of filtrable viruses kept this question to the fore. Can even more minute corpuscular bodies exist or can life even exist in a dissociated state as a chemical unit without clearly defined corpuscular limits?

As long as we are dealing with visible phenomena, our knowledge is direct, but when we attempt to study infravisible forms of life, even with the microscope, conclusions must be inferential.

This is true of what we know of the characteristics and activity of bacteriophage just as it is true of our very definite knowledge of the structure of the atom, of electrons and the like.

D'Herelle has concluded that bacteriophage is a living corpuscular element of about the size of a colloidal micella, that it enters the bacterial cell at a single point multiplying therein and after having increased about eighteen times intracellularly, disrupts the bacterial cell, after which the liberated bacteriophages are free to enter new hosts.

Bacteriophage is quite ubiquitous, being present in the intestinal tract of animals, in water, and in soil, but for growth it requires the presence of the bacterial cell. In bacterial cultures it grows, reproduces, and is transplantable in series. This would not be the case were it an inanimate chemical. As small an amount as one hundred billionth part of a cubic centimeter has been found sufficient to cause the dissolution of a suspension of bacteria or of bacterial culture.

With bacteriophage implanted into a susceptible bacterial culture, after thirty minutes of contact the bacteriophage corpuscles have almost entirely disappeared from the liquid. After sixty minutes the situation is the same. After ninety minutes the corpuscles have suddenly reappeared in the liquid, and their number is eighteen times greater than was that of the inoculated corpuscles. In other words each inoculated corpuscle has yielded eighteen. D'Herelle produces evidence that during the interval of one and one half hours the phage is attracted to the bacterial cells, perhaps by chemotaxis, that it enters the cell at one point, that it multiplies therein, and finally ruptures the cell almost with explosive violence.

As the phage continues to grow, it does so not in a continuous, progressive fashion but by sudden successive increments, these sudden jumps being explicable on the hypothesis that in the intervals the bacteriophage is intracellular.

The diameter of a single bacteriophage has been estimated at between twenty and thirty millimicrons. This is essentially the same diameter as that of the protein micella.

Strains of bacteriophage have varying degrees of virulence for bacteria, and the latter may require a resistance against invasion by the former. The virulence of bacteriophage may be enhanced for any of a large variety of bacteria by repeated passage through these bacteria as a pabulum. Twenty five bacterial species have already been found susceptible to bacteriophages.

The bacteriophage apparently is an electronegative protein colloid. Its resistance to destructive agents is almost as great as is that of bacterial spores. Whatever its nature, this element possesses those characteristics which we usually consider as associated with life. These are the power of assimilation in a heterologous medium and of adaptation, the power of multiplication, and a variability of characteristics. The fact that the bacteriophage corpuscle has been demonstrated to be a simple protein micella and that it is a living being show that the cellular concept is erroneous. Life results from a particular physical chemical state of the protein micella. There appears to be but one species of bacteriophage. There is an unlimited number of races, the special characteristics of each being acquired by adaptation.

The normal habitat of the bacteriophage is the intestinal tract, but it may pass into the circulation and into the tissues without detectable disturbance. After parenteral introduc-

¹The Bacteriophage and its Behavior. By F. d'Herelle, M.D. Translated from the French by George H. Smith, Ph.D., Associate Professor of Bacteriology and Immunology, School of Medicine, Yale University. Cloth, Pp. 629. Published by the Williams and Wilkins Co., Baltimore, Md.

tion the bacteriophage corpuscles behave like the spores of saprophytic bacteria. They disappear quickly from the circulation and are last found in the liver and spleen. The action of bacteriophage in disease, such as bacillary dysentery however, depends upon the virulence of the particular bacteriophage which happens to be present against the dysentery bacillus. Phagotherapy has given very promising results in the treatment of the bacillary dysenteries in staphylococcus infections, and in bubonic plague.

These are but the high lights. There is a wealth of interesting material in d'Herelle's monograph. The translation made by George H. Smith is of particular excellence.

Hunter Tod's Disease of the Ear

SINCE Dr Tod's death Dr George C. Cathcart has edited and rewritten this well known British textbook on the ear. The opening chapters deal with the physics of acoustics, the anatomy and embryology of the ear, the physiology of the ear and the theories of hearing. There follows a chapter on methods of examination which while not discussing the more unusual tests or those of doubtful value, describes clearly and concisely those generally used. The remaining chapters deal with diseases of the auricle, the external canal, the middle ear, the mastoid and with intracranial disease of otitic origin. This includes disease of the internal ear and the auditory nerve. There are also chapters on deaf mutes and diseases of the nose and nasopharynx in their relation to diseases of the ear.

Surface Equilibria of Colloids†

DUNOY'S monograph deals with his researches in physical chemistry and the physics of colloids at the Rockefeller Institute. The major portion of his book will be of interest chiefly to physical chemists, biologists and physicists for it is highly technical. However, the concluding hypothesis which he presents as a result of his studies is of interest to all students of life.

Until comparatively recently the conception of life was cellular and the doctrine *omnis cellula ex cellula* was generally accepted. At least twenty years ago however Victor C. Vaughan and others hypothesized that life may exist in more minute form than the cells as we know them today. The experiments of d'Herelle on bacteriophage show concrete evidence that this assumption is probably correct. The observations of various workers particularly French workers, on the granular form of the tubercle bacillus contribute to the same end. Dr DuNouy enables us to understand how cells may be formed from protein material which originally was noncellular. He concludes that this is primarily a matter of the surface equilibrium of colloids. The most probable configuration of colloidal equilibrium is the cell form. The dynamics of surface tension cause a concentration of protein at the surface of a droplet such that the surface layer becomes 300 or 400 times more viscous than is the interior.

The entire work is of great interest but this conception of the original development of the cell during the evolution of life is probably the outstanding feature of the author's work.

Pernicious Anemia‡

THE color index in a secondary anemia, such as anemia due to hemorrhage, is low because the red blood cells are replaced easily and quickly while a longer time is required for the regeneration of iron containing hemoglobin. The high color index in pernicious anemia and

Hunter Tod's Diseases of the Ear. Revised and largely rewritten by George C. Cathcart, M.A., M.D., Consulting Surgeon to the Throat Hospital, Golden Square. Late Member of the Special Aural Board, Ministry of Pensions. Second Edition. Illustrated. Cloth. Pp. 333. Humphrey, Milford, Oxford University Press.

†Surface Equilibria of Biologic and Organic Colloids. By P. Lecompt, DuNouy, D.Sc. with introductions by Dr. Alexis Carrel and Prof. S. O. Robert A. Millikan. Cloth. Pp. 131. The Chemical Catalogue Company, Inc. New York. 1916.

‡Pernicious Anemia. By Frank A. Ryan, M.D. Cloth. Pp. 15. The Williams and Wilkins Company. 1916.

in some of the acute infections and in the later stages of prolonged infections may be explained by the lowered functional activity of the blood forming organs in the presence of a normal or adjusted iron metabolism. A high color index does not necessarily mean that the anemia is primarily hemolytic, due to increased destruction of blood, but indicates only an anemia of long duration and slow development. In anemia, particularly in pernicious anemia, the destruction of blood takes place within the body and the iron is conserved. Pernicious anemia is a disease of adult life, probably never occurring earlier than the fourteenth year and being decidedly infrequent in the aged.

While the pathologic picture in pernicious anemia is varied and there are many incidental findings such as the blood changes, the absence of hydrochloric acid in the gastric contents, the deposition of iron containing pigment in the reticuloendothelial cells, and fatty degeneration of the muscles and parenchymatous organs, there are certain pathologic changes which can only be explained as dependent on some as yet unknown cause. These are fatty degeneration of the viscera and muscles, erythroblastic changes in the bone marrow, the marrow, and focal degeneration in the white matter of the spinal cord.

We do not know the final cause of pernicious anemia but several suggestive facts are available. The close analogy between this disease and the anemia associated with *Dibothriocephalus* latus infestation, sprue, and the so called pernicious anemia of horses encountered in Central Europe (due to the presence of larvae of certain flies within the stomach), all suggest a possible infectious or parasitic etiology with the consequent absorption of a hemolytic toxin.

Again it has been suggested that pernicious anemia is a manifestation of chronic anaphylaxis resulting from the absorption of foreign proteins from the bowel. A highly suggestive explanation is that tyramin will produce in guinea pigs an anemia closely resembling that of pernicious anemia. Tyramin has been isolated from putrifying meat and can be produced from tyrosin by the action of certain bacteria. The colon bacillus is in this group. Thus, a powerful hemolytic agent may be produced within the intestine. The normal habitat of the colon bacillus is the lower intestinal tract. It has been suggested that when for some reason it invades the upper intestine it becomes a harmful organism. Here it would have greater opportunity to act upon the incompletely digested proteins. Some cases of pernicious anemia have been found to possess strictures in the intestine. Experimental strictures in dogs, five or ten centimeters above the ileocecal valve, sometimes produce what appears to have been a hemolytic anemia. In those dogs in which this occurred the bacterial flora of the small intestines was the same as that in the colon. In those dogs in which no anemia resulted the flora remained normal.

Red blood cells of normal animals which have passed through the spleen and are obtained from the splenic vein are found to be less resistant to hemolysis than are those in the systemic circulation. Removal of the spleen increases the resistance of red cells to hemolysis. These findings suggest that the spleen weakens the resistance of the red cells and that this is a factor in the hemolytic anemias—that they may be due to a hypersplenism. While this is possibly true, it may not be a factor of great importance in pernicious anemia for here the resistance of the red cells to hemolysis is increased rather than diminished. Furthermore clinical improvement does not constantly follow splenectomy.

In hemolytic anemia there is an increase in the degree of unsaturation of the fatty acids of the blood. Oleic acid shows greater hemolytic activity than does stearic acid which has a lesser degree of unsaturation. Lamar has shown that the hemolytic power of a fatty acid or its soluble soap varies directly with its degree of unsaturation. Removal of the spleen lowers the degree of unsaturation of the fatty acids of the blood, and the result is an increase in the total fat and cholesterol. Cholesterol under some circumstances is a powerful antihemolytic agent. Thus, after removal of the spleen we observe a diminution in a hemolytic agent and an increase in the quantity of an antihemolytic agent in the blood.

However, there is much contradictory evidence and it must be recognized that the exact rôle of the spleen in hemolytic anemias is not clearly known. Probably many extrasplenic factors also play a part. Turk has said "The hemolytic diseases are the children, and the spleen is their mother, but the father is still unknown, and possibly there are several fathers."

The hemolytic anemias may not necessarily be due to an increase of hemolytic substance in the blood but equally well to a decrease in any antihemolytic substance which should be present. The total amount of cholesterol, an antihemolytic substance in the blood of patients with pernicious anemia has been shown beyond question to be lower than that in normal blood.

Blood cells appear to take an active part in lipid metabolism and where their number is diminished each assumes a larger share of the work there being apparently an increased concentration of lipid and lecithin in the red cells. Possibly in anemia the hemolytic fatty acids which are normally held in the blood plasma in combination with cholesterol are taken up in greater quantities in the red cells with consequent destruction of the latter. The two tissues which contain more lecithin than any other in the body are the envelopes of the red cells and the sheaths of the nerves. The two outstanding changes in pernicious anemia are the hemolytic anemia and a diffuse sclerosis of the spinal cord. Lysis of the erythrocyte appears to be dependent upon the presence of certain lipid substances within the cells.

Whatever the ultimate cause of pernicious anemia may be found to be it seems probable that some disorder of splenic function and of the lipid metabolism of the body will be found to be of pertinent interest.

Dr. Evans' volume on Pernicious Anemia is essentially practical and covers the subject from various clinical angles. He discusses the usual routine methods of treatment going into considerable detail with transfusion and its results and mentions other procedures which have shown more or less promising results such as iodine treatment and cholesterol feedings. The reviewer recommends particularly the author's discussion of the mechanism responsible for good results after transfusion. This can no longer be considered merely the replacement of lost blood. The resultant biologic reactions are of far deeper significance.

*Hydrogen Ion Concentration of the Blood in Health and Disease**

THE authors describe this small volume as intended as a convenient outline for those readers whose interest has been clinical rather than physiologic. The reviewer feels that those clinicians who will read the volume in the hope of finding therein a simple clear cut readily intelligible discussion will be in great measure disappointed. We have read discussions of the subject which, while no more elementary are decidedly more easily understood by one whose knowledge of hydrogen ion concentration is not great. The authors employ complicated chemical equations in profusion and apparently believe that they will be readily understood by all readers. More detail might have been devoted to the development of these equations. Or, if the book is intended particularly for the clinician they might better have been avoided in so far as possible. A specific example of the lack of clearness to refer to only one is in their discussion and explanation of the Donnan theory of membrane equilibrium.

In great measure the monograph is a review of literature. Throughout the discussion there are frequent references to the work of others with only brief recapitulative remarks on their results.

On the other hand if one is willing to settle down and dig out the information contained in the volume, he will find much of interest and of value.

Hydrogen Ion Concentration of the Blood in Health and Disease. By J. Harold Van den Broek, Professor of Research Medicine, University of Pennsylvania; and Glenn L. Cullen, Professor of Biochemistry, Vanderbilt University Medical School. Cloth. Pp. 70. The Williams and Wilkins Company. 1936.

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EDITORIALS

Yeast-Like Fungi and Pernicious Anemia

TWELVE years ago Ashford,^{1, 2, 3} in a series of papers embodying a large series of observations, advanced the suggestion that sprue, a chronic catarrhal inflammatory condition of the gastrointestinal tract, was due to infection with a specific yeast-like fungus, *Monilia psilosis*

These findings were corroborated by some^{4, 5, 6} but denied by other investigators^{7, 8, 9} and the more recent attitude of Ashford concerning the etiology of sprue is in accord with that of the majority of authorities that it is a symptom complex produced by glandular insufficiency upon which is superimposed a gastrointestinal infection with *Monilia psilosis*¹⁰

Somewhat later, Wood¹¹ emphasizing the clinical similarity between sprue and pernicious anemia, reported the finding of *Monilia psilosis* in the stools of 15 patients and then absence in 40 controls, stated that a blood picture similar to that of pernicious anemia could be produced by feeding the fungi to guinea pigs, and suggested, therefore, a possible etiologic relationship of *Monilia psilosis* to pernicious anemia

This new candidate for etiologic fame in pernicious anemia did not long

remain unchallenged. The experimental production of anemia by feeding failed to be confirmed by Warthin¹² and by Broun,¹³ and Baumgartner and Smith¹⁴ demonstrated *Moulinia* in only 21 per cent of cases of pernicious anemia while finding it in 29 per cent of cases of diarrhea.

A rather extensive series involving the culture of 192 stools from 121 individuals and 31 gastric contents from 29 individuals have been recently reported by Nye, Zervas and Cornwell¹⁵ and serves to assist in the clarification of the confusion and divergence of opinion concerning not only the importance of yeast like fungi in the intestinal tract, but also in regard to the classification of isolated strains.

Particular interest attaches to their findings because of the correlation of clinical and cultural data.

Among the cases studied were 18 cases of pernicious anemia in which the stools were cultured, the gastric contents being cultured in 7. Ten normal controls were examined and the remainder of the cases included a variety of pathologic conditions, among them carcinoma and tuberculosis.

The results of this investigation are thus summed up by the authors:

1. Cultures of stools and gastric contents from a variety of diseases have yielded numerous strains of yeast like fungi, by far the majority of which belong in a single species group, *Parasaccharomyces A*.

2. The members of this large group cannot be distinguished from *Moulinia psilosis* (six of the strains isolated were so classified by Dr. Ashford), nor from yeast like fungi isolated from typical thrush membranes or sputum, and are apparently of common occurrence in the human gastrointestinal tract.

3. The two outstanding features of the correlation of cultural and clinical data are: a, a higher incidence of yeast like fungi in the gastric contents of gastric achylia, and b, the failure to isolate species, groups other than *Parasaccharomyces A*, in appreciable numbers from the stools.

4. The percentage of isolations of *Parasaccharomyces A* from the stools and gastric contents in pernicious anemia were no greater than those in cases of severe anemia and gastric achylia other than pernicious anemia from which it is concluded that *Moulinia psilosis* is unimportant as an etiologic factor in either sprue or pernicious anemia.

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already topheavy list, means "strange disease" and all students of the subject certainly agree upon this point although Coca and Cooke proposed primarily for hay fever and asthma in which heredity is a frequent and important factor.

The outstanding fundamental change would appear, therefore, to be a state of *altered reactivity* of the body cells which may be in the usual sense of exaggerated susceptibility or the reverse of reduced susceptibility or tolerance. Accordingly it would appear that the term "allergy" is most appropriate of all proposed for designating this broad and important subject, although it conveys no idea at all of the mechanism concerned but merely emphasizes the characteristic and outstanding change of an altered reactivity of body cells which in the usual sense is one of exaggerated susceptibility.

That immunity to diphtheria and other diseases also involves the questions of susceptibility and tolerance of body cells, that is, of a change in their reactivity is entirely irrelevant to the subject, as the mechanisms are fundamentally different with a complete independence of the true allergic phenomena from the ordinary bacterial antigen-antibody reactions as shown by Zinsser and his associates.

But if this is an acceptable term for the whole subject, upon what basis should subclassifications be made? Are we justified in classifying on the basis of being able or unable to demonstrate antibody in the blood? Or according to what is known of the chemical nature of the antibody? Or according to the influence of heredity?

I have long objected to any classification based upon our ability or inability passively to transfer the antibody and especially to guinea pigs and rabbits, because failure to do so could not be accepted as indicating the absence of antibody. Indeed only a few years ago it was believed that the allergic antibody to timothy and ragweed pollens could not be passively transferred to guinea pigs until this possibility was demonstrated by Walzer and Grove by improved methods. And now that we have the Prausnitz-Kustner method of passive transfer to human skins this objection would appear to be still further justified, since it has shown the presence of antibody in the serum of human beings with various allergies not only to horse serum but also to various pollens, pea and other foods, and to dandruffs and other exciting agents of asthma which could not be transferred to the lower animals. It is true, however, that all attempts to transfer passively drug, bacterial, and poison ivy allergies by the guinea pig and Prausnitz-Kustner methods have so far failed, but this does not necessarily mean that antibodies are absent. It may merely indicate failure to demonstrate their presence in the circulating blood. In such instances they may be attached to the sensitized cells as so-called "sessile receptors" as is apparently true at least in the case of tuberculosis.

Memory. Indeed I believe it can be stated without reserve as a widely established fact that the allergic reaction is a cellular phenomenon and under some circumstances I see no escape from the possibility, and indeed the probability, that in some allergies the antibody is predominantly cellular with hypersensitiveness as the type, while in others it is predominantly

humoral, with serum hypersensitiveness as the type, although we are totally unable to explain at present why there should be this difference in the distribution of the antibody. Possibly experiments consisting of the extraction of the sensitive epithelium of the skin of individuals with tuberculin, ivy, and drug allergies will demonstrate the presence of antibody in the cells and at any rate it cannot be said that antibody is absent, we are merely justified in stating that its presence in the blood has not been demonstrated. Therefore, my objection to any classification on this basis.

But may allergy be classified on the basis of the chemical nature of the exciting agent or whether or not it is antigenically active? Personally I believe that a classification on the basis of the chemical nature of the exciting agent is an acceptable one at the present time. And by this method we would have but two groups namely, (1) those in which the exciting agent is known to be protein or closely related to it and including not only serum but the foods dandruffs probably the pollens, and even tuberculin and the products of other bacteria and (2) those in which the chemical nature of the exciting agent is unknown as in the case of the drug poison ivy, and similar plants causing dermatitis venenata. It is true that the exciting agent of pollens and house dust have been regarded as being nonprotein in nature by Grove and Coca, Black and others, but more recent studies by Walzer and Grove have shown that some at least may be protein, a recent study in my laboratory with ragweed pollen has convinced me that this is a fact, while the work of Long and Scribner and of Zinsser and Tamura have shown that the active substance in tuberculin and other bacterial allergies is a protein or closely related to it. Here again we must be very cautious in stating that the exciting agents of allergy are nonprotein on the basis of being nondigestible and nondialyzable when it is recollected how minute an amount of agent can elicit an allergic reaction and readily escape chemical identification. Indeed I am fully prepared to see proved that the exciting agent of poison ivy is a protein or closely related to it and likewise in the case of quinine, morphine, belladonna oils, balsams resins and other drugs of vegetable origin although this possibility can be excluded in the case of arsenic, mercury, iron, coal tar derivatives and other metallic drugs unless they really do combine with body proteins to form an antigenically active foreign protein as originally suggested by Wolff Eisner. The latter, however, has never been proved but neither has it been definitely disproved. When one remembers that the predominant thought in the mechanism of parasitropism in chemotherapy involves a change in the chemical nature of arsphenamine and other compounds in the blood or other tissues following administration for the production of new parasitocidal compounds, it must be admitted that this idea of Wolff Eisner's is not to be lightly thrown aside but wisely left open for future investigation. Indeed I know of no more important field for research in allergy than upon the mechanism of drug hypersensitiveness with special reference to the possible role of antibody and the chemical nature of the exciting agents.

Furthermore, the fact that a substance is unable to induce the production of demonstrable antibodies, like precipitins and agglutinins in the blood, that is, fails to be antigenically active in the immunologic sense does not necessarily mean that it is unable to induce allergic sensitiveness. It means nothing more than that it fails to produce antibodies in the blood but leaves wide open the possibility of producing antibodies attached to cells as in the case of tuberculin allergy. As a matter of fact I think it can be stated at present that there is no relation whatsoever between precipitins in the blood and the allergic antibody. Several investigators have shown that the antibody may be present in a sensitizing serum in which precipitins could not be demonstrated and that, conversely, sera containing large amounts of precipitin, like rabbit antiegg and rabbit antiragweed, were unable to produce allergic sensitization even by the sensitive Prausnitz-Kustner method, although it is possible that a repetition of these experiments with changes in technique may result in positive reactions. Also that the allergic antibody may be neutralized in the test tube without any visible precipitation or alteration in the specific activity of the latter as shown by Coca and his associates. So why make any classification on this basis? It is true that precipitin and allergic antibody are frequently present together, but in my opinion we may safely accept as a fact that they are not identical and that precipitin is unnecessary in the mechanism of allergy.

And was it necessary to coin the new word reagin for designating the antibody in hay fever and asthma in view of the already long list of names for the antibody concerned in the allergic reaction? Certainly the antibody in serum allergy may be acquired by immunologic stimulation and furthermore the so-called "reagin" concerned in the Wassermann reaction, which led Coca and Grove to adopt the term in relation to hypersensitiveness, is indeed an antibody of the kind active in the test tube even though without demonstrable effect upon *Spirocheta pallida* and probably heterophile in nature. Furthermore tuberculin and other bacterial allergies may be acquired by immunization as shown by Zinsser and his associates and the frequency with which the symptoms of hay fever and asthma first appear in adult life, the acquisition of primrose sensitiveness by Bloch, etc., has led me to the belief that these may be acquired by frequent contact with the exciting agents despite the negative outcome of animal experiments. Indeed I believe that altogether too much emphasis has been laid upon the outcome of experiments with guinea pigs and rabbits in studying this question of acquired allergic sensitization, since they are by no means as susceptible to either active or passive sensitization as are human beings except in the case of serum allergy.

Furthermore, I doubt the wisdom of insisting upon successful transfer of antibody by the Prausnitz-Kustner method as a criterion of asthma or other allergic states since the presence of free or circulating antibody need not be present if its cellular situation is the important change. In other words this
it may have a greater positive than negative value, and in my opinion a

negative result is not acceptable as evidence of the absence of possible allergic sensitization due to a strictly cellular situation of antibody

Indeed Fineman has expressed the opinion that the degree of sensitiveness of asthmatics is better elicited by skin tests than by determining the antibody content of the blood although Levine and Coca have observed that skin sensitiveness in early and late hay fever bore a relation to the antibody content of the blood in almost all cases, and Clark and his associates have recently advocated the passive transfer method as being probably more reliable for judging the clinical severity and danger of serum allergy than skin tests. All of this leaves one with the impression that allergic sensitiveness may exist without demonstrable antibody in the blood and that while skin tests may be more valuable for diagnosis, the presence of circulating antibody may be more dangerous or at least more significant of acute and even dangerous sensitization in human beings.

This question of the relative clinical importance of fixed or cellular and free or circulating antibody, however, is still further complicated by the results of treatment of hay fever and asthma since there may be an increased toleration or amelioration of symptoms without appreciable decrease in either skin sensitiveness or of allergic antibody in the blood. This leaves us at present very much in the dark regarding the mechanism of desensitization, although I am confident that future investigations with more accurate methods will show a quantitative decrease of sessile or cellular antibody and fully justify the present use of the term "desensitization" as indicating a reduction of sensitization of body cells by exhaustion of antibody. It also leaves us in doubt about the meaning of the term 'anaphylaxis' which is applied to the refractory state supposed to be due to the presence of sufficient antibody in the blood to neutralize effectually small amounts of exciting agent or allergen in the blood before the latter reaches sensitized cells with the production of shock even though this circulating antibody may afford no protection against the local or topical application of the allergen to the sensitized cells of the skin or a mucous membrane. Since circulating antibodies have been found with such regularity in hay fever, asthma and serum allergy by the Prausnitz Kustner method without demonstrable protection of the affected individuals it is apparent that the exact mechanism of this state and of non specific desensitization are as yet unknown and deserving of reinvestigation for elucidation.

And is a classification warranted on the basis of hereditary influence? No one can deny that heredity plays an important role in hay fever and asthma, but it must be remembered that it likewise plays an important role in infection and immunity to disease in general as indicated by the clinical studies of Draper and Stockard and the laboratory studies of Lewis and Loomis in tuberculosis of guinea pigs whose work has indicated a possible correlation between antibody production and allergic irritability.

In the first place what is the nature of this hereditary influence? All that is definitely known is that the majority of cases of hay fever and asthma have one or more antecedents with some type of recognized allergy and that there

is transmitted a tendency to develop allergic sensitiveness of some kind without, however, any direct transmission of any particular allergy by either mother or father, although Clark, Donally and Coca have recently stated that the offspring of hay fever subjects are more likely to suffer from hay fever than bronchial asthma, and the reverse. Does this mean the transmission of a particular tissue or shock organ concerned in the production of allergic antibody? If so, it has not been demonstrated or proved. Or does it mean the transmission of a peculiar tendency or facility for producing the antibody, that is to say, of tissues tuned up as it were for the production of the antibody? This likewise is unproved, although a far more attractive thought and possibility. Or does it mean the transmission of a peculiar sensitiveness to the effects of allergy as I proposed in 1923? This, too, has not been proved, but I believe the possibility is worthy of consideration and study.

From the clinical standpoint I have always been impressed with the importance of vasomotor instability or irritability in hay fever, vasomotor rhinitis, asthma, and other human allergies. These individuals blush very easily and are sometimes very sensitive to changes in temperature, scratches, etc. Furthermore, it is commonly and widely accepted that vascular changes, and especially dilatation, congestion and edema, are the outstanding and prominent tissue changes because the allergic shock organ or tissues in human beings is largely involuntary muscle. Under these conditions it is reasonable to believe that individuals with sensitive vasomotor systems, that is, individuals who show an exaggerated tendency to localized or generalized vasomotor disturbances would be more susceptible to the effects of allergy than the more stolid individuals who possess less sensitive vasomotor systems and are less sensitive to vasomotor disturbances. In my opinion the transmission of an unduly sensitive vasomotor system to any irritant or emotion is the hereditary factor in allergy and that it is possible that many human beings actually acquire allergic sensitiveness as to milk for example, without showing signs or symptoms simply because they are less susceptible to the effects of allergic shocks. Certainly human beings are much more susceptible to allergy than the lower animals and are much more likely to develop or present vasomotor disturbances. Furthermore, young children are much less likely to show hay fever and asthma than adults, and it is well known that they have better balanced vasomotor systems. Moreover, hay fever and asthma are less commonly observed among negroes than among whites and they, too, as a class have less sensitive vasomotor systems than the latter, and finally, hay fever, especially, is more likely to occur among high-strung individuals of both races than among the more stolid and lowly. All of this has led me to regard a sensitive vasomotor system as the hereditary factor, not with any peculiar or specific relationship to allergy, but simply one of greater susceptibility to the effects of allergy because smooth muscle and particularly the smooth muscle of blood vessels happens to be an important shock organ in human allergic reactions. I have no objection to the coinage of atopy as a special designation for those allergies in which heredity can be traced, because all

allergies are indeed strange diseases, but I think it was uncalled for in an already overburdened nomenclature and especially since more intensive studies may show a similar influence of heredity in more clinical types of allergy than are now surmised.

These considerations led me in 1923 to propose in the third edition of my book on *Infection, Immunity and Biologic Therapy* (page 598) a classification in which the whole group of phenomena were designated under the common term of allergy with two subgroups namely (1) those allergies caused by protein exciting agents or those closely related to proteins and which in the light of our present information may include natural and acquired serum sensitiveness, hay fever, most forms of asthma, tuberculosis and other forms of bacterial sensitiveness and (2) those caused by drugs of nonvegetable origin possibly ivy sensitiveness, and other forms of dermatitis venenata if it is finally proved that the exciting agents of the latter are toxic glucosides and definitely non protein in nature. If, however it is subsequently shown that drugs and ivy form protein compounds in the tissues this classification would be unnecessary and any further classification be for clinical purposes and convenience only in the way of giving separate names for clinical entities of basically similar mechanism. I see no reason at present for abandoning this classification, indeed and on the contrary, I believed that the results of investigations since 1923 have still further shown that it is both reasonable and substantially correct.

It may be that in the future we shall remove drug allergy altogether from the group of allergic conditions since it is included now almost solely by reason of the fact of its resemblance to allergy but with almost no further justification in view of our present information on its mechanism. Indeed we commonly overlook the fact that drug sensitiveness and tolerance may be more of a pharmacologic than an allergic problem and more than enough harm and confusion have been done in medicine by the power and tyranny of analogy. For example, in chemotherapy we have more than begun to abandon the theory of chemoreceptors of Ehrlich, proposed to explain organotropism or toxicity for the body cells and parasitropism or toxicity for the parasite on the basis of the existence of hypothetical specific side chains or receptors analogous to the obsolete side chain theory of immunity. Rather I am personally in favor of placing the whole business upon a definite chemical theory in which the chemical compound does or does not combine with body cell or parasite, according to whether the protoplasm of the latter does or does not contain chemical constituents of physiologic importance possessing a combining affinity for the chemical compound or medicament. It may be that in so called drug idiosyncrasy the body cells happen to contain naturally or acquire as the result of former administrations more of the chemical constituent possessing an affinity for the drug than the body cells of the average and majority of individuals without involving the question of possessing a superabundance of theoretic chemoreceptors and that acquired drug tolerance is not a matter of exhaustion of receptors or allergic antibody but merely a matter of exhaustion of the chemical constituent of the protoplasm having this combining affinity for the chemical agent or

drug If this mechanism is finally proved, the drug idiosyncrasies and tolerances could be removed from the category of allergy, but in the meantime we may leave it in this group with the idea in mind that it is possible that a protein exciting agent is produced by union of drug and body proteins, that an allergic antibody to this drug-protein antigen is produced which is entirely cellular in situation and that drug tolerance involves a mechanism of exhaustion of this cellular antibody in the same manner as is probably operative in the desensitization to other allergies caused by agents of known protein constitution and mediated by demonstrable antibody

In conclusion I believe that in the interests of orderliness and clearness it is possible, advisable, and permissible to get rid of many terms which now contribute so materially to confusion and with this in mind I advocate the adoption of a simpler terminology as follows

- 1 To employ the term *allergy* and abandon that of anaphylaxis, hypersensitiveness, idiosyncrasy, and atopy

- 2 To designate the exciting agent as *allergen* and abandon the terms anaphylactogen, sensibilogen, sensitinogen, and atopen

- 3 To base classification upon what is known of the chemical nature of the exciting agent and drop classifications based upon the success or failure of demonstrating antibody in the blood by passive transfer of sensitization to guinea pigs or human skins or according to success or failure of active sensitization of guinea pigs Or to base it upon a division into clinical types, as the dividing line between acquired and natural allergies is too poorly defined for classification on these grounds as is likewise a division into so called normal and abnormal allergies

- 4 To designate the antibody as *allergen* or allergic antibody and abandon the terms anaphylactin, sensitizin, reaction body, albuminolysin, precipitin and reagin

- 5 To adopt the cellular theory in its essentials as the underlying mechanism and abandon the humoral and related theories

- 6 To adhere to the terms active and passive sensitization because less cumbersome, more euphonious and better known than the term allerization

- 7 To adhere to the term desensitization for expressing a decrease of symptoms under therapeutic intervention as a result of increased toleration for the exciting agent even though its exact mechanism is unknown

It seems to me that this simplified nomenclature is quite sufficient for present purposes, at least until it is proved that the phenomena in man and in the lower animals involve separate and distinct mechanisms with different kinds of antibody But in my opinion this has not yet been proved, and I believe the term allergy is ample for designating the phenomena as a whole, as likewise the term allergen for designating the wide variety of exciting agents, allergin for the antibody, etc., as long as we have reasons for believing that the mechanism of serum allergy in the guinea pig is fundamentally the same as serum allergy, hay fever, asthma, etc., of human beings

THE POTENTIAL ASTHMATIC*

By F. M. POTTINGER, M.D., MONROVIA, CALIFORNIA

CLINICAL PICTURE OF ASTHMA

WHEN one sees a patient suffering from a paroxysm of asthma the dyspnea as a rule overshadows all other symptoms. On examining the patient, however, if the case is severe, the physician notes a rapidity of heart action, a cyanosis, a diminished respiratory excursion, a tense emphysematous chest moving in the inspiratory phase. Rales and rhonchi are heard of a character more or less distinctive, which together with the particular character of the respiratory murmur with its prolonged expiratory phase have come to be recognized as making up the asthmatic type of breathing. If the attack is severe and prolonged, the heart may be enlarged and show signs of embarrassment. There may be a considerable or a small quantity of sputum. Eosinophilia is usually present. The condition may have developed suddenly or slowly.

HOW DOES ASTHMA EXPRESS ITSELF?

Asthma expresses itself in the neurocellular mechanism of the bronchus as a contraction of the smooth musculature, an exudation into the tissues, and an increase in bronchial secretion. This we at once recognize as a vagotonic or parasympathicotonic picture.¹

The neurologic picture, however, is not dependent on nerve stimulation alone. There is a primary cellular element in every physiologic or pathologic action, which of course is modified by various hormones as well as the nervous system. Biophysicists have proved that nerve action depends upon the physical state of the cell, its degree of alkalinity or acidity, also, its content as well as that of the surrounding fluids in substances such as nutritive materials, cholesterol hormones and various electrolytes such as *Ca*, *Na*, *K*, *P*, and *Mg*.^{2, 3, 4, 5}

It is further shown that the vagus which is the parasympathetic component of the vegetative system, concerned in the production of asthma, is closely associated in its action with the *K* of the cell. Without *K* there can be no normal parasympathetic action. In parasympathicotonia we have a tendency to deficiency of blood calcium and excess of blood potassium with alkalosis and increase of blood phosphates.

Asthmatics are very apt to show endocrine disturbances particularly that of hypoadrenia, hypothyroid, hypoparathyroid and hypogonad, and the thymicolympathic state.

The vagus is opposed in its action in the bronchial musculature by the sympathetics, which are linked up in their activity with the *Ca* of the cell.

*Read before the Sixth Annual Meeting of American Association for the Study of Allergy Minneapolis June 11-12 1929

Therefore we have two antagonisms in the normal or physiologic function of the bronchi, the sympathetic and vagus nerves in the correlating mechanism, and the *Ca* and *K* of the cell in the production of the fundamental activities of tonus and rhythm

We have a third antagonism of a chemical nature the *H* and *OH* of the tissues. These also align themselves as antagonists, the *H* with the sympathetics and *Ca* and the *OH* with the vagus and *K*

Like all schemes of action these are subject to variability and, at times, effects the reverse of what are expected are observed. There are times when stimulation of the vagus nerve may produce acceleration of the pulse and when an increase in *Ca* may be followed by a parasympathetic instead of a sympathetic effect, so these antagonisms must not be accepted as universal and unalterable, but rather as being the usual relation, the one met most often under normal circumstances

That asthma is a local parasympathetic syndrome cannot be questioned. That it is often met in individuals who show universally increased parasympathetic tonus or parasympathetic syndromes in certain organs other than the lungs is also well known. Bradycardia, epiphora, vasomotor rhinitis, hay fever, nasopharyngeal catarrh, spasmophilia, hypermotility and hypersecretion of the gastrointestinal canal, spastic constipation, mucous colitis, diarrhea, shell-fish poisoning, urticaria, angioneurotic edema, eosinophilia, digestive hypoglycemia and hypotension are found frequently in the group of patients to which asthmatics belong

CAUSES OF ASTHMA

In our discussion of the cause of asthma we must approach it from a double point of view, first, that of the potential asthmatic, and second, that of the precipitating factor

A certain percentage of people, so-called constitutional arthritics, are potential asthmatics at birth and as soon as they contact stimuli which are directed toward the pulmonary parasympathetic neurocellular mechanism, they may develop the asthmatic syndrome. Many such individuals develop asthma soon after birth, others, in the early years of life. There are still others who, although they have a very unstable equilibrium between the sympathetic and parasympathetic bronchial neurocellular mechanism, yet, under ordinary circumstances remain free from asthma. When subjected to unusual parasympathetic stimulation, however, members of this group, too, may develop the asthmatic syndrome. During childhood such individuals often are subject to colds, sneeze on slightest exposure, are subject to hay fever, laryngitis, spasm of the glottis, and bronchitis. Slight colds may be accompanied by sonorous rales in the chest. These conditions are at once recognized as being of a catarrhal congestive and exudative type such as has been suggested as belonging to the exudative diathesis. Many of these individuals during childhood, as well as in later years, are subject to eczema and urticaria. These, with other parasympathetic syndromes previously mentioned, make up a distinct type of vegetative imbalance which belongs to the vagotonic constitution

It seems that in the group possessing these particular constitutional characteristics, there occurs at times an incomplete elaboration of protein, which results in unmetabolized products gaining access to the blood and lymph streams, from which they are deposited in the skin and mucous membranes. These products possess the property of sensitizing tissues, and particularly of producing their effects upon the parasympathetic neurocellular mechanism. When the substances which have sensitized the cells again come in contact with them a reaction of hypersensitivity occurs. Such a reaction may appear as a vasomotor rhinitis, a hay fever, an asthma, a severe gastrointestinal disturbance, an urticaria, an eczema, a colloidoclastic reaction or other syndromes which are less easily recognized.

Certain individuals of this group who are prone to asthma also seem to possess constitutional hepatic inadequacy. This is of great importance because of the detoxicating influence of the liver upon substances of alimentary origin. Such individuals show idiosyncrasy to foods and suffer from anaphylactic states more often than those with normal liver function. They also show hypoglycemia which aligns them with the vagotonic group.

Constitutional vagotonia, speaking of the condition as an inherited one is as a rule widespread although its manifestations may be confined to certain tissues or organs so we must think of asthma as being a local manifestation of a more or less generally unbalanced neurocellular mechanism.

A hypersensitive neurocellular mechanism is not only prone to be rendered allergic to protein but this hypersensitivity may be expressed in a heightened reaction to any and all stimuli. An instance of this is the vasomotor rhinitis which is associated at times with gastrointestinal disorders and with sexual congress also asthma which results from reflex stimuli arising in other organs such as the nose, heart, gall bladder, gastrointestinal tract, and pelvic organs.

Hypersensitive tissues and organs also react readily to local stimuli of an irritating character and to general stimuli such as those of an emotional nature and those due to weather changes. The fundamental cause of the reaction in all of these conditions is not the particular exciting factor but the constitutional instability which makes the neurocellular mechanism hypersensitive to stimuli of many kinds. Therefore antigens, chemical substances, changes in weather and reflex impulses acting upon hypersensitive cells produce paroxysms of asthma.

I personally had an experience which illustrates how local cells may become specifically sensitized. I have always been subject to vasomotor rhinitis and hay fever. One time I was taking a tablet of aspirin for headache. When I had chewed the tablet to a powder I was compelled to sneeze and this forced some of the powder into my posterior nares. Almost instantly the tissues became edematous and closed the nares completely, a condition which remained for several hours. The edema was accompanied by a profuse nasal discharge. Since that time whenever I take aspirin a vasomotor rhinitis occurs. This condition is analogous to the specific allergic reaction which

follows the deposition of pollen in, the tissues of the mucous membrane of the nose in the production of hay fever, and the tissues of the mucous membrane of the bronchi in the production of asthma

TREATMENT OF ASTHMA

If the conditions herein mentioned underlie asthma and hay fever, then one or more of the following therapeutic measures suggest themselves first, to change, if possible, the parasympathetic neurocellular hyperirritability, second, to desensitize the cells if specifically sensitized, third, to remove the patients to surroundings which are free from substances to which they are sensitized, in case of air-borne stimuli, and to withhold from use by the patient foods or other substances to which they are known to be allergic, and, fourth, to remove known sources of reflex stimulation

The success of desensitization of the body cells as a method of relieving asthma has been due in no small measure to the energy of members of this Society Their individual contributions rank with the best that have been made to the subject The administration of thyroid extract in certain instances, when a deficiency of this hormone was present has proved that in some instances the affection possesses endocrine aspects The correction of reflex irritation arising in other organs has been successful sufficiently often to show this as a factor The same may be said of climatic change

All of these measures, however, leave much to be desired They leave most patients still potential asthmatics, waiting for the proper stimulus to induce another attack The probable reason for this is that they fail to change or correct the constitutional or acquired hyperexcitability of the bronchial neurocellular mechanism and so leave the patient in a condition to again become asthmatic when the exciting stimulus arises Potential asthmatics possess different degrees of hypersensitivity It is a common experience that some are much easier to relieve than others Where the imbalance is very marked most measures have so far proved unavailing

Could the tendency to congestion and exudation in the tissues be relieved and the constitutional parasympathicetonia be overcome, probably asthma and the entire group of allied hypersensitive conditions could be averted So far, however, we do not know how to cure it, but we do know certain measures that improve the condition of the imbalance Parasympathicetonia is not a matter of hyperirritability of the nerves alone It is a complex condition in which the tissues which react with the parasympathetics also have a very important part Relief of parasympathetic syndromes may be brought about by forces which depress parasympathetic nerve action, or by forces which stimulate the antagonistic sympathetic neurons, or, by decreasing the substances which are responsible for activity in parasympathetic structures, or by increasing the substances which produce inhibition in them

In this connection hormones must be considered The successful use of adrenalin in relieving asthmatic paroxysms proves its relationship definitely, but there are other hormones whose relationship to the sympathetic action is also very close, and which must be considered Such are the thyroid, parathy-

roid, and testicular hormones. In appropriate cases hormones from these glands may be used advantageously for their therapeutic effect along with other antiasthmatic measures.

Atropine is a substance which lowers parasympathetic activity, particularly that due to the vagus nerve. The cell acted upon by the nerves, however, is just as important in parasympathetic hyperactivity as the nerves themselves. These too may be influenced therapeutically as is evident from the antagonistic action of Ca and H ions in the cells.

Calcium may be able to combat parasympathetic syndromes in several different ways. In tissues innervated by both sympathetic and parasympathetic neurons such as the pupil, the nasal mucous membrane, the bronchi, the heart, and the gastrointestinal tract, calcium acts with and fortifies sympathetic activity, and in so doing opposes the parasympathetic action. Calcium, further, has a property of allaying muscular hyperactivity as we see in tetany and in spasms in various tissues. It further decreases the permeability of cell membranes, in which it antagonizes sodium and potassium. By so doing it decreases the tendency to exudation in the tissues.

Such effects should all be of value in influencing the underlying condition which is responsible for asthma. That Ca does have such effects is shown in many parasympathetic syndromes met in clinical practice. Its effect in relieving the intestinal spasm, productive of pain and diarrhea in tuberculosis of the bowel is well recognized. Its relief of urticaria and the anaphylactic phenomena accompanying serum reaction is often gratifying. In hay fever and asthma it will often give great relief.

A disappointing experience in the use of calcium is that one cannot always produce the desired effect in overcoming the spasm, even though its employment is based on sound physiologic principles. This, however, must be expected. Physiologic reactions differ from test tube reactions in that there are often individual differences in patients and differences in the same patient at different times which interfere with always obtaining the same reaction following what seems to be the same stimulus. At certain times a reaction occurs directly the reverse of the one expected. Such has been noted by physiologists in experimental animals, where under certain conditions stimulation of the vagus produces acceleration of the heart instead of inhibition. In still other cases we may not be able to overcome the parasympathetic overbalance, no matter what remedy we use.

The employment of calcium has been considered of no avail by some because of the findings on blood calcium estimation. Patients with asthma have been examined and found at times to have the usually accepted normal or even increased amounts of Ca in the blood. Again it has been shown that after Ca administration, usually within three hours the blood calcium returns to its former level. In regard to these observations it can be said that the normal Ca content of the blood varies under different circumstances and in different individuals. Environmental conditions undoubtedly cause variation in

the blood *Ca* The amount of sunshine and seasons are known to influence it So do various hormones So must we expect a variation in the *Ca* content of the blood of asthmatics

It is not the blood calcium alone that is to be considered but the *K Ca* ratio, the relative tonus of the sympathetic and parasympathetic nerves, the hormone balance, and the *H OH* ratio Klyn⁶ found on determining the *K/Ca* ratio of the blood of a large group of individuals that in normal persons the ratio is approximately 2, there being twice as much *K* in the blood as there is *Ca* In asthmatics, on the other hand, the ratio was always high, running from 2.04 to 2.93, averaging 2.48 In only one case did it fall below 2.19

Hietenyi⁷ has shown that while the blood calcium attains a normal level within a few hours after calcium administration there is still a retention of about one-half of the *Ca* administered somewhere in the body tissues Of 260 mg administered, after three hours, there was still an increase of 6 mg in the blood, while 35 mg had been eliminated in the urine and 90 mg in the feces Thus 131 mg could be accounted for, leaving 120 mg unaccounted for but somewhere in the body This indicates that a low serum-calcium content may mean either a reduction of body calcium or a storage in the tissues

Calcium must not be administered in severe asthmatic paroxysms in the hope of relieving the paroxysm, as may be done by adrenalin, but for a slow change in cell activity Its action may be increased by the simultaneous administration of atropine

SUMMARY

1 There are two causes of asthma, the fundamental underlying cause and the precipitating cause

2 Asthmatics belong to the constitutional arthritic class, and particularly to those of the exudative diathesis

3 Asthma expresses itself as a local parasympathicotonia, usually in an individual who shows more or less general parasympathicotonic phenomena

4 Constitutionally, asthma is associated with an overbalance of the parasympathetic nerves, an overbalance of *K* as compared with *Ca* in the cells, an increase in *OH* ions as compared with the *H* ions of the tissues, and a deficiency in certain sympatheticotonic endocrines, such as the adrenals, thyroid, parathyroids, and gonads

5 Sensitization to food, pollens, etc., response to reflex impulses from other organs, also to chemical and mechanical irritation, and sensitiveness to weather changes, are only precipitating factors in a disorganized neurocellular mechanism

6 Treatment must be directed not only to the relief of the precipitating cause but also to changing whenever possible the hypersensitive neurocellular mechanism

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DISCUSSION

Dr Warren T Vaughan Richmond Va—This paper should receive a lot of discussion I feel that the Society is particularly fortunate to have such a paper to open the meeting with We are prone to become rather one sided in our outlook on the subject of allergy and to talk of it too much in terms of protein sensitization So for a keynote speech, we have a paper which says very little about protein sensitization

We fail to recognize, or at least to emphasize often, that asthma is more or less of a chemical reaction in which two bodies react It is not only the allergen which is responsible but it is also the reacting body which is the human being So we must consider both sides of the chemical equation

I have a lantern slide I want to bring out one or two points Perhaps the first point is that in our discussions of the subject of immunology or allergy we have so many different terms applied to one and the same phenomenon or process which makes it difficult sometimes for some of us to understand the other fellow's language We talk about immunology, allergy, and atopy and everyone has to slip in a new term I have been guilty of attempting to slip in a new term myself so I feel that I can voice the criticism

(Slide See chart in article on Role of Specific and Nonspecific Factors in Allergy and Allergic Equilibrium JOUR. LAB AND CLIN MED April, 1928 in, 633)

This is a table which I have elaborated more for my own good than for anyone else, because I feel that I can visualize fairly clearly the underlying processes and can explain it to patients very easily with the help of this chart

"The specific allergenic causes of allergy and the nonallergenic causes" There is much that could be criticized in this chart, for example as to whether bacteria are specifically allergenic or not, and in the second bracket we find the groups which Dr Pottenger is speaking about particularly He is talking about the type of individual We must consider both the reacting body the specific allergenic factor, and the individual The second bracket I feel, includes a great deal of what Dr Pottenger has so clearly described to us

The first bracket is divided into two sections Certainly I think most folks will agree that a specific allergen is responsible for most outbreaks of allergic disease On the other hand there are cases in which we cannot find a specific protein or allergenic cause so that it is conceivable that these two reactions may occur to produce an allergic attack without the intervention of the first or specific series of causes

Allergic equilibrium or balanced allergic state is another term for the vagotonic predisposition which Dr Pottenger was speaking about, and that is where I must apologize for having attempted to bring in another term that means the same thing essentially which Dr Pottenger has discussed The rest of the chart does not enter into the present discussion

Of course heredity is probably the most important predisposing factor We may possibly have an allergically predisposed individual caused by liver insufficiency The French have talked long about deficient gastric leucopoiesis They measure it in this way The gastric contents usually contain a variable number of leucocytes which have passed through the stomach wall into the stomach and they quantitate these leucocytes, after the ad

ministration of a peptone solution. They find that in asthmatics, there is often a diminished number of leucocytes in the stomach contents after stimulation caused by the ingestion of peptone solution.

That is going to be of some interest in the near future because of the recent work that has been done, first, on the use of nitrohydrochloric acid in the relief of hay fever, and second, the observation which was reported at the May meeting of the Society for the Study of Asthma, that achlorhydria often accompanies allergy.

I shall not go into further detail. I want to bring out the second bracket of my chart in confirmation of Dr. Pottenger's discussion and emphasizing the fact that there are two factors to the allergic reaction.

Dr. F. M. Pottenger, Monrovia, Calif.—I have nothing further to say. I am very glad to see Dr. Vaughan's chart.

Of course, terminology is one of the very difficult things in medicine, and it is particularly difficult when we are taking up new subjects of this kind, but it is impossible for any of us to jump right at the end of things when we begin.

I used the word "parasympathetic" in preference to vagotonia simply because I am trying to get the whole study of the vegetative nervous system on a definite basis. The vagus nerve does not include all of the parasympathetic nerves, so I think it is very much better if we speak of a person suffering from sympathicotonia and parasympathicotonia, although the words are quite long.

The thing that has interested me in asthma has been this: Why does a person become asthmatic? Why does one individual react to pollen, or to other allergens? Why does he react to stimulations of various kinds, and why does he react to various weather changes, and so forth, where another person is not bothered at all? That has been the purpose of my study more than anything else. I think we must take it into consideration with Dr. Vaughan's chart. I think he has a very splendid expression here.

We are very apt to look at all subjects, not only in medicine, but everything else, from too narrow a standpoint. It is very hard to be broad. It is very hard to get a conception of the other fellow's point of view. Medicine is made up of so many specialties today that it is almost impossible for anybody to talk on any one subject intelligently. My reason for bringing this paper before you was not in any way to oppose the idea of allergy, but simply to call attention to something more fundamental upon which your allergy is acting, and that brings up the fundamental characteristics of the individual and his particular methods of reaction. I think we must study those things.

In medicine we must go back to heredity. We have to take up the question of disposition as we have never attempted before. We are entering on the physiologic era. I think the next move is to begin this study of physiology with the instructive studies which have already been made on psychology, physiology, neurology, and the physics of the cell as they alter the patient's individual reaction. I do not know any place where it is more necessary to know this than it is in the study of the asthmatic, because the asthmatic is an individual who gives an entirely different type of reaction from other ordinary diseases in which the physiology of the individual is probably the fundamental factor.

PATHOLOGY OF ASTHMA, NONBACTERIAL ALLERGIC AND BACTERIAL TYPES BASED ON AUTOPSY MATERIAL*

By BERNHARD STEINBLERG M.D. AND H. D. FULEY M.D. TOLEDO, OHIO

THE pathologic changes occurring in people affected with asthma are not well defined either in the mind of the pathologist or of the clinician. This lack of clarity is due to the questionable etiology and paucity of the post mortem material. Asthma is seldom the direct cause of death. Out of a thousand cases, Rackemann¹ reports five deaths and an autopsy on only one of them. The literature contains studies of twenty five cases. Huber and Koessler collected fifteen and presented six of their own. Lemaire, Leon, Kindberg and Levesque² reported one. Rackemann¹ one and Delmer³ two cases. All the authors, except Rackemann classify their cases under the term of bronchial asthma. Since asthma merely means a type of dyspnea with wheezing sounds in the chest and the term bronchial refers to the involvement of the bronchi, this symptom complex was used to designate pathologic changes in what was assumed to be a distinct disease entity. Thus Schmidt⁴ considers his case that of bronchial asthma. His patient developed asthmatic attacks three weeks prior to death and the autopsy revealed a malignant growth occluding pulmonary arteries and bronchi. One of Fienkel's cases⁵ was a man of sixty three who had rheumatism and gout for years. He had bronchial catarrh for three years and one year before death developed asthma. The autopsy revealed a dilated heart and emphysematous lungs. Tikhmeneff's⁷ patient, although having asthmatic attacks for eleven years, died of empyema, pulmonary abscess and pneumonia. Ellis⁸ from the study of his own case and those in the literature concluded that the pathologic anatomy of bronchial asthma is not the same in all cases though in some points they closely correspond. It is apparent that it has been attempted to establish a pathologic entity for a symptom complex produced by several distinct conditions.

Meltzer's⁹ association of bronchial asthma with anaphylaxis, the introduction of the allergic theory, and Waller's¹⁰ concept of sensitization by bacterial proteins have somewhat clarified our knowledge of bronchial asthma. However, until the etiologic factor or factors are definitely determined, the employment of the term bronchial asthma, irrespective of etiologic evidence lends confusion to an already confused subject and makes it difficult to establish a pathologic entity.

In a number of the twenty five reported cases the asthmatic attacks were preceded by bronchitis or sinus infections. Berkhart's¹¹ patient had been subject to frequent colds since childhood and the first attack of asthma followed

*From the Division of Laboratories and Research and the Department of Medicine, Toledo Hospital, Toledo, Ohio.

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a prolonged bronchitis. One of Huber and Koessler's² patients had a severe bronchitis for six months prior to the first attack of asthma. One of our cases had sinus infection and bronchitis prior to the appearance of asthma. It is the consensus of opinion that there is a type of asthma which is preceded by an infectious process of the sinuses, respiratory passages, or both. For this condition, we are employing the term bacterial asthma which is now in frequent use. Whether the asthmatic attacks are due to sensitization by bacterial proteins or to some other factors is irrelevant so far as the employment of the term is concerned. The pathologic anatomy of this type of asthma can be differentiated from the other type to be discussed.

In another group of the twenty-five cases, there was no evidence of any infectious process. There was usually present a familial history of asthma. If due to pollen, the attacks were more or less seasonal. If foods or animal emanations were responsible, removal of the offending factors relieved the patient. Cutaneous tests not infrequently gave positive reactions to extracts of pollen, foods, or animal emanations. This type of asthma we are indicating as nonbacterial allergic asthma.

Of the twenty-five cases reported, only eight actually died from asthma. Rackemann¹ analyzed Huber and Koessler's twenty-one cases and found only four whose deaths were due to uncomplicated asthma. The additional four cases were those of Rackemann, Lemierre, Leon-Kindberg and Levesque, and Dehner. We are adding to the literature two more cases: one of nonbacterial allergic asthma dying during an asthmatic attack and another case of bacterial asthma.

NONBACTERIAL ALLERGIC ASTHMA

Clinical History—Female, aged sixty, married, American, was first seen by one of us (K. D. F.) at Toledo Hospital, April 23, 1927, suffering from frequent severe attacks of asthmatic dyspnea.

Family History—One sister had asthma. Brother's child has had Fall-type of hay fever for several years.

Previous History—Married forty years. Had given birth to two children at seventh month of gestation and neither child survived. First asthmatic attack began at her home at Archbold, Ohio, at the age of forty-two. This attack followed mowing of the lawn. Since then, asthmatic attacks have occurred the year round, but always worse and more frequent during the summer months. These attacks were often preceded by sneezing, coryza, lachrymation and burning of the eyes. Of late years, the patient noted some dyspnea on exertion, which was relieved by rest. This dyspnea occurred independently of her attacks of asthma.

Present Illness—The asthmatic attacks began at her home the latter part of March, 1927. These attacks occurred daily and several times during twenty-four hours, and were usually worse at night.

Physical Examination—Woman of medium build. Slight cyanosis of lips and nails. The mucous membrane of nose was pale and there was polypoid change of right middle turbinate. The sinuses were clear on transillumina-

tion, corroborated by a competent rhinologist and by roentgenogram. The chest was somewhat barrel shaped. The heart was of normal size, the rhythm was regular but the rate persistently rapid. During attacks there were wheezing sounds in the chest with a lengthened and labored expiratory phase. The blood pressure averaged 140/80. The electrocardiogram showed tachycardia, low amplitude of curves in all leads and a suggestion of right ventricular preponderance. Examination of the abdomen, extremities, and reflexes gave normal findings.

Blood. Hemoglobin 76 per cent, red blood cells 3,910,000, leucocytes, 8,800, polymorphonuclears 79 per cent, lymphocytes 19 per cent, eosinophiles 2 per cent. Urine, amber, acid, specific gravity 1020, trace of albumin, few white blood cells. Blood sugar 84 mg per 100 cc of blood, blood nonprotein nitrogen 36 mg, blood urea 7.8 mg, blood calcium 9.8 mg, blood Wassermann negative. No sputum could be obtained.

Cutaneous tests on April 25, 1927, gave mildly positive reactions to ostrich feathers, rabbit hair, sheep wool, and three tree pollens. There was a suggestive positive reaction to an intradermal injection of feather extract. On May 7, 1927, other scratch skin tests gave mildly positive reactions to numerous tree, grass, and weed pollens.

Treatment and Course—The patient was under observation in the hospital from April 23 to May 11, 1927. During this time she had repeated asthmatic attacks which were relieved by small doses of adrenalin. At no time was there coughing or expectoration either preceding or following the asthmatic attacks. About a week before her death, there occurred several days of very high winds during which time the patient was worse—the asthmatic attacks being almost continuous. Pollen plates in the window of the patient's room showed the presence of enormous amounts of tree and grass pollens. After three days and nights of especially severe asthmatic seizures, the patient died. Death was apparently due to respiratory failure as the heart remained regular and the systolic blood pressure was maintained at a level of 130 up to death.

Clinical Diagnosis—Nonbacterial allergic asthma, probably due to pollen.

Points in the case report that point to an allergic basis for the asthmatic attacks. The onset of the original attack in summer following cutting grass, the attacks more frequent and worse in summer, the attacks formerly preceded and accompanied by symptoms of hay fever, history of allergic manifestations in other members of the family, allergic appearance of nasal mucosa, positive cutaneous tests to numerous tree, grass, and weed pollens as well as a few animal emanations, extremely severe asthmatic attacks prior to death coincident with presence of large amounts of tree and grass pollens in the air.

PATHOLOGIC REPORT

Macroscopic Description—The body is that of a well developed and moderately well nourished, white adult woman. The shape of the chest is suggestive of a half barrel. The abdomen is protuberant. There is slight edema of

the lower extremities and cyanosis under finger nails. The liver extends 5.5 cm below the costal margin. The heart weighs 275 gm, is brown in color and shows a decrease in the subepicardial fat. The left ventricular wall measures 14 mm, the right ventricular wall 8 mm, the left auricular wall 3 mm and the right auricular wall 1 mm. The right ventricular wall shows penetration of yellow streaks from the subepicardial fat into the musculature. The right lung weighs 300 gm, and the left 240 gm. The lungs contain large areas of bulbous emphysema and are reddish-yellow in color. The cut surface is dry, spongy, and red-yellow. The trachea and large bronchi have a red mucosa which is covered with a red, shiny material. The bronchioles, the small and medium sized bronchi contain white, firm plugs, which obliterate the lumen completely in the greater number of tubes. These plugs cannot be dislodged even by firm pressure. The spleen weighs 135 gm, the cut surface is purple-red and the knife scrapes away very little of the pulp. The connective tissue is increased. The liver weighs 1230 gm. The edges are sharp. At the junction of the superior and anterior surfaces are two ridges 4 mm in width and 3 cm in length. Both kidneys weigh 270 gm. The capsule strips without difficulty leaving a smooth, nongranular surface containing fetal lobulations. The left kidney, in the cortex and medulla of the upper pole, contains an encapsulated, grey-yellow, elevated, soft mass, 4 cm in diameter. The brain shows moderate edema. The other organs do not show any significant changes.

Microscopic Description—The heart shows, in the right ventricular wall, separation of muscle bundles by “signet-ring” cells. The muscle cells contain a moderate amount of bipolar brown pigment. The blood vessels are congested. The spleen contains thickened blood vessels. The follicles are smaller than normal and contain small areas of a homogeneous pink staining (eosin-stained) amyloid. The sinuses are distended with blood. The mass in the upper pole of the left kidney consists of tubules lined by cuboidal epithelium. The epithelium is nowhere outside the basement membrane. The tubules are confined by a connective tissue capsule. The lungs show the greater number of the alveoli distended and intercommunicating through broken-down septa. The alveolar lumina are clear. The interalveolar tissue contains dilated and congested capillaries and blood vessels. The bronchioles, small and medium sized bronchi, are dilated, the lumina are entirely or partly occluded by mucus which contains desquamated epithelial and few mononuclear cells, some of which are eosinophilic. The mucous material is adherent in many places to the epithelium. The lining cells are everywhere intact. The basement membrane is hyalinized in the greater number of bronchi and bronchioles. The subepithelial tissue is edematous and is infiltrated by a large number of mononuclear cells, many of which are eosinophilic. The muscle layer is markedly thickened. The mucous glands show absence of nuclei, obliteration of cell outline and a hypersecretion of mucus. The muscle layer and the glands are infiltrated by mononuclear cells many of which are eosinophiles.

SUMMARY OF THE PATHOLOGIC FINDINGS IN THE LUNGS

Complete or partial obliteration by mucous plugs of the lumen of most of the bronchioles, small and medium sized bronchi, hyperplasia hypertrophy and hypersecretory activity of the mucous glands hypertrophy of the muscle layer, edema and cellular infiltration of all the coats a large number of eosinophiles in the cellular exudate emphysema



Fig 1—Nonbacterial allergic asthma. Cross section of bronchi small and medium size and lung tissue. a Lumen of bronchus completely filled by mucous material b muscle layer shows hypertrophy c the mucous glands are hyperplastic hypertrophied and show hypersecretory activity d lung tissue showing emphysematous areas (x10)



Fig 2—Nonbacterial allergic asthma. Cross section of bronchus in Fig 1 under higher magnification. a Lumen of bronchus completely filled with mucous material and cells the lumen is distended and tortuous b hypertrophied muscle layer c hyperplastic hypertrophied and hypersecretory mucous glands d cartilage (x30)

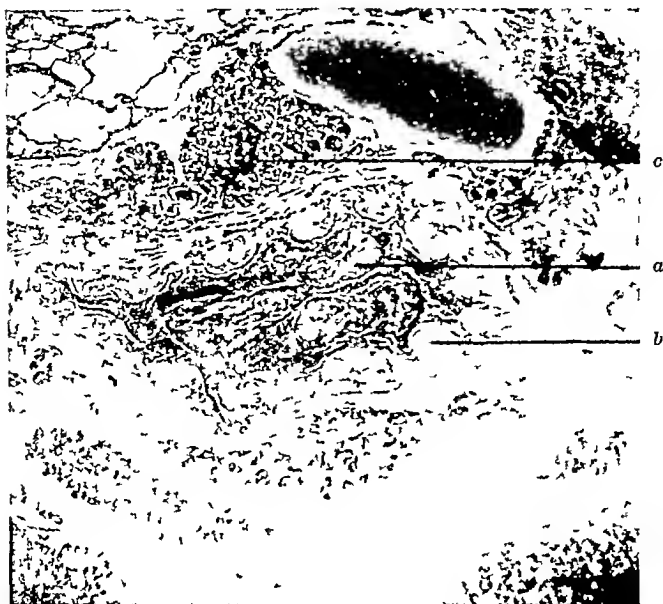


Fig 3—Nonbacterial allergic asthma Cross section of bronehus of 2 mm inside diameter *a*, Lumen of bronchus completely filled with spirally arranged mucus *b* hypertrophied circular muscle bundles *c* mucous glands showing hypersecretory activity hyperplasia and hypertrophy (x45)

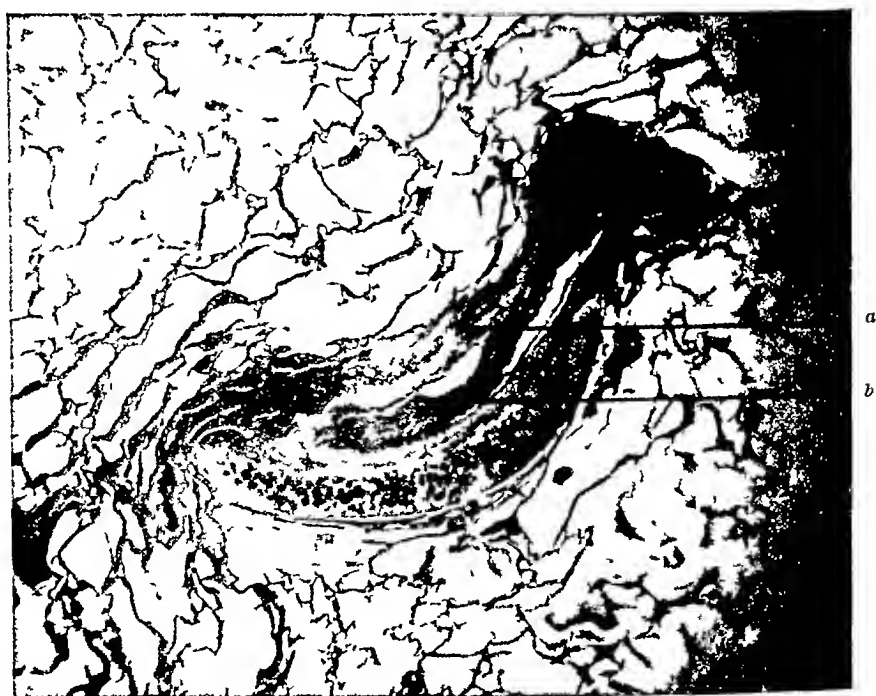


Fig 4—Nonbacterial allergic asthma Cross-section of bronchus 1 mm inside diameter *a*, Greatly hypertrophic muscle layer *b*, lumen almost completely filled with mucous material (x45)

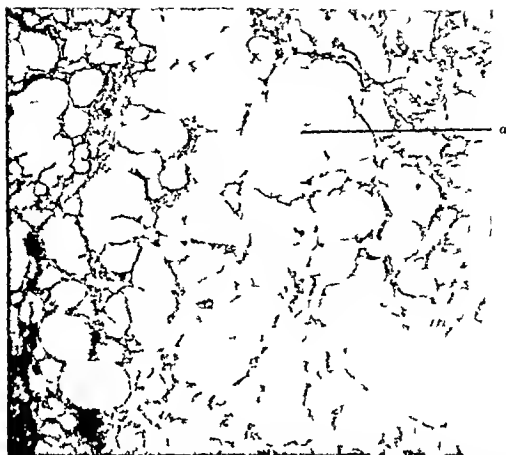


Fig 5—Nonbacterial allergic asthma. Cross section of lung showing emphysema. *a* the alveoli are distended the interalveolar walls are broken through and several alveoli intercommunicate (x45)

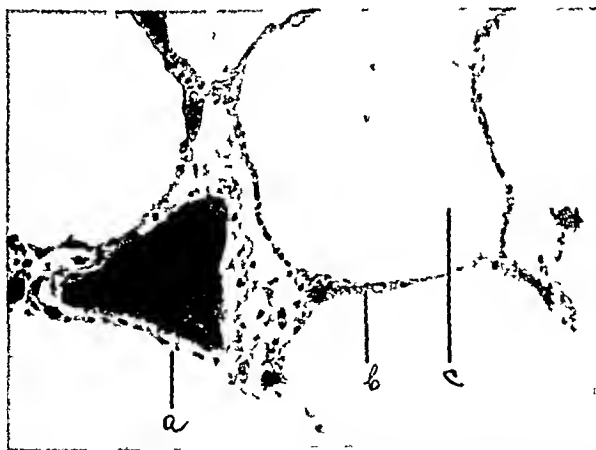


Fig 6—Nonbacterial allergic asthma. Cross section of lung with emphysema. An area from Fig 5 under higher magnification. *a* Blood vessel is distended and filled with blood *b* distended capillaries filled with blood *c* dilated alveolus

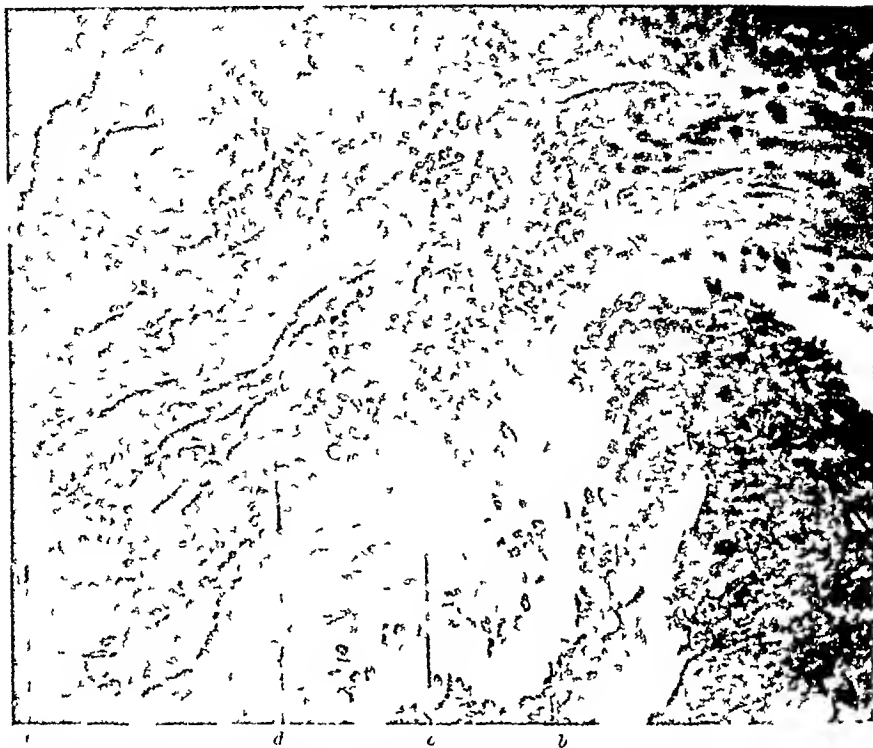


Fig 7—Nonbacterial allergic asthma. Cross-section of a part of bronchus with an inside diameter of 3 mm. a Lumen of bronchus filled with mucoid material which contains desquamated epithelial cells and eosinophils; the mucus is firmly adherent to the lining epithelium. b hyalinized subepithelial tissue. c hypertrophic muscle layer diffusely infiltrated by mononuclear cells many of which are eosinophilic. d eosinophilic. e lymphatic filled with coal pigment ($\times 600$)

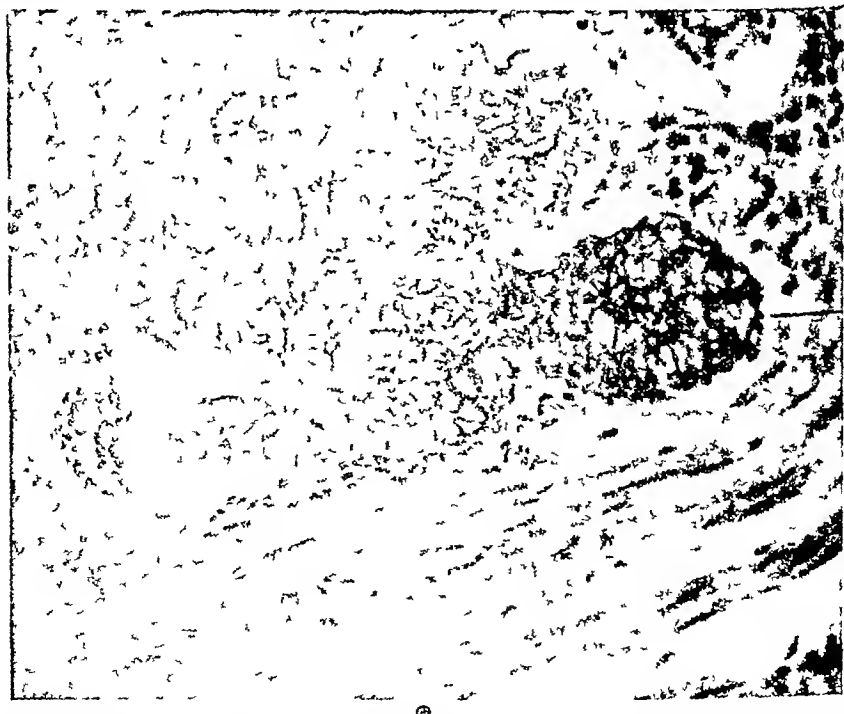


Fig 8—Nonbacterial allergic asthma. Section of part of bronchus showing a part of the muscle layer and the mucous glands. a The mucous glands show hypertrophy, loss of nuclei and cytoplasm and a large amount of mucous secretion. There are many mononuclear cells infiltrating the glands, some of these cells are eosinophils ($\times 500$)

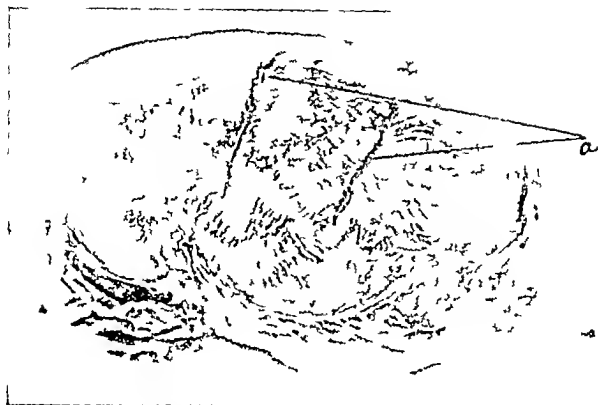


Fig 9—Nonbacterial allergic asthma. Gross photograph of the liver. Two notches on the superior surface due to pressure of diaphragm as a result of hyperinflated lung.

Pathologic Diagnosis—Chronic emphysema of lungs, mild chronic obstructive bronchitis, brown atrophy of the heart, fat infiltration of the heart, tubular adenoma of the left kidney, edema of the brain.

BACTERIAL ASTHMA

Clinical History—Mrs E A H, aged sixty-one married Swiss American, was first seen by one of us (K D F) in July, 1922 suffering from severe paroxysms of coughing and asthmatic dyspnea.

Family History—Husband has arrested pulmonary tuberculosis. Three daughters are living and well. There is no history of allergic manifestations in the family.

Previous Medical History—Typhoid fever at the age of twelve with complete recovery. Married at the age of twenty-five. Mother of three healthy living children. Always well since attack of typhoid until present illness.

Present Illness—In May, 1922 patient had a severe attack of so-called "influenza." This was followed by paroxysms of coughing which were usually unproductive and severe enough to provoke vomiting. Eventually these coughing spells led to the asthmatic type of dyspnea. The dyspnea and cough became progressively worse. On August 5, 1922, patient was first admitted to Toledo Hospital.

Physical Examination—Woman of fifty-five with evident respiratory distress, coughing frequently and leaning forward with each paroxysm of coughing. Some cyanosis of face and lips. Nails cyanotic. Nasal mucosa showed evidence of a chronic inflammatory process and there were polypi in right upper nasal fossa. Frontal sinuses and the antra were clear on transillumination. This was corroborated by roentgenogram and by a competent rhinologist. Chest showed physical signs of emphysema. Throughout both lungs

there were numerous dry and moist sibilant râles of all degrees of intensity. Breathing was of the asthmatic type with a lengthened and difficult expiratory phase. The heart was impossible to outline on physical examination but the roentgenogram showed slight enlargement to the left. The rate was somewhat increased but the rhythm was regular. Blood pressure was 140 systolic, 80 diastolic. The liver edge was palpable two fingers' breadth below the costal margin, there were no other abnormal abdominal findings. Examination of reflexes, glands, extremities gave normal findings.

Blood Hemoglobin 80 per cent, red blood count 4,200,000, white blood count 10,000, polymorphonuclears 75 per cent, lymphocytes 20 per cent, eosinophils 5 per cent. Urine amber, specific gravity 1021, acid. Wassermann negative. Cutaneous tests All negative to various foods, pollens, animal emanations, house dust, etc.

Treatment and Course—During this admission, a large polypus containing green, mucopus was removed from the right upper nasal fossa by Dr E G Galbraith, who expressed the opinion that it originated from chronic ethmoid infection. Under rest and sedatives, the asthmatic dyspnea gradually subsided, especially after the expectoration of large amounts of thick, greenish sputum was well established.

Following this admission, the patient was seen at home several times during the late fall of 1922. She had severe attacks of asthmatic dyspnea and paroxysmal cough. These attacks were always made worse by the slightest exertion and were relieved by the raising of thick, green, mucopurulent sputum. In February, 1923, the patient developed broncho-pneumonia and was admitted to Toledo Hospital. There was again much asthmatic dyspnea with raising of thick sputum. During this admission, an electrocardiogram showed some myocardial impairment with numerous extrasystoles. On December 11, 1924, the patient was again admitted to Toledo Hospital, having had several attacks of asthmatic dyspnea at her home, since her last admission. These attacks usually followed "taking cold" and were relieved when expectoration was freely accomplished. During this admission, Dr Galbraith irrigated the left maxillary sinus with prompt relief from the distressing cough and dyspnea. Roentgenogram of the sinuses showed abnormal changes in the ethmoids and in the left maxillary antrum. In March, 1927, following the usual succession of asthmatic attacks at intervals of several weeks to months, the patient was again admitted to Toledo Hospital. This time, the dyspnea was relieved more quickly by ephedrin. The roentgenogram showed no change in the heart shadow as compared with previous examinations. The antia were clear. Urine and blood showed normal findings. Blood urea nitrogen was 15 mg, CO₂ combining power 68. Sputum culture showed a pneumococcus type IV. Wassermann was again negative and repeated cutaneous protein tests were negative. On February 15, 1928, the patient was again seen at her home with the complaint of cough and severe headache. She stated she had not felt well for three days and thought she had "taken cold". She became unconscious shortly after and was removed to Toledo Hospital where she died on February 23, 1928. During her stay in the hospital, she was almost com-

pletely anuric, and had intractable vomiting, and several convulsions, which started with twitchings of the left arm and leg. Leucocytes 25,000 with 87 per cent polymorphonuclears. Blood nonprotein nitrogen 120 mg, urea nitrogen 75 mg, creatinin 4 mg. Spinal puncture returned a normal fluid under normal pressure. Urine showed much albumin, granular and epithelial casts, numerous white blood cells and red blood cells.

Clinical Diagnosis—Bacterial asthma (asthmatic bronchitis) death due to bacteremia from sinus or bronchial infection acute nephritis

PATHOLOGIC REPORT

Macroscopic Description—The body is that of a well developed well nourished, white, adult woman. The nose shows excoriation and deviation of the septum to the left. There is pyorrhea alveolaris. There is moderate edema of the extremities and cyanosis of finger nails. The upper lobe of the right lung is adherent by firm fibrous adhesions anteriorly and posteriorly. The lungs fill completely both pleural cavities. The liver extends for 4 cm below the costal margin. The heart weighs 380 gm. There are small subepicardial hemorrhages. The right lung weighs 630 gm, the left lung 435 gm. There is moderate emphysema, more marked in the middle and lower right lobes. In the emphysematous areas, the lung is crepitant, elsewhere it is rubbery in consistency. A creamy pus is expressed on pressure from the cut surface. The trachea, the large and middle sized bronchi show a red mucosa covered by yellow purulent material. The smaller bronchi are filled with yellow pus. The pulmonary arteries show occasional elevated areas of intimal thickening. The lymph nodes are large and anthracotic. The liver weighs 1130 gm. The edges are round. The cut surface shows deep red central zones surrounding the central veins. The combined weight of both kidneys is 410 gm. The cortex measures 8 mm. The striations are parallel and regular. The glomeruli are red. The brain shows diffuse hemorrhages in the right cerebral cortex. The sphenoid and ethmoid sinuses are filled with a yellow grey material. The posterior nares contain yellow pus. The mastoid cells and middle ears show no abnormal changes.

Microscopic Description—Heart shows diffuse subepicardial hemorrhages. The capillaries are distended and congested. There is some fragmentation of myocardial cells and patchy replacement of muscle by fibrous connective tissue. The splenic capsule is increased in thickness. There is a greater than normal amount of fibrous connective tissue. The sinuses are distended with blood. Liver shows necrosis of central zones with a diffuse infiltration of the necrotic areas by red blood cells. The kidney shows replacement of cortical areas by connective tissue with presence of few lymphoid cells. Some glomeruli are completely fibrosed. The subcapsular space in a number of the glomeruli contains albuminous material. The capillary tufts are distended with blood and show an increased cellularity. The tubules especially the proximal convoluted, contain hyaline casts albuminous material and red blood cells. The brain shows an infiltration of the vessel walls by polymorphonuclears and mononuclears. In places, the vessel walls are ruptured and red

blood cells are seen in the break and in the surrounding brain tissue. The cortex of the brain shows in places pyknosis and karyorrhexis of nuclei and destruction of cell cytoplasm. An occasional blood vessel shows an embolus consisting of bacterial masses and cells. Lungs show large patches in which the alveoli are obliterated, many of these areas consist of connective tissue. The bronchioles and the bronchi of the smallest caliber, between 0.5 and 1 mm inside diameter, show within the lumina a cellular exudate consisting of broken and intact polymorphonuclears and mononuclears. The lining cells are absent in many places, in other places, there is metaplasia from columnar to squamous epithelium. The walls are diffusely infiltrated by similar cells. There is only an infrequent eosinophile. An occasional small bronchus shows obliteration of the lumen by connective tissue. The muscle layer of the bronchioles and of the small bronchi is moderately hypertrophied. The mucous glands show no change except for a moderate infiltration by mononuclear and polymorphonuclear cells. An occasional cartilage shows a bone marrow reaction. The medium and the larger sized bronchi show within the lumina only a small amount of a cellular exudate but the walls contain foci as well as a moderate infiltration of polymorphonuclears and mononuclears.

Summary of the Pathologic Findings in the Lungs—Atelectasis, cellular exudate filling the lumina of the bronchioles and small bronchi, destruction of the lining cells of the mucosa, diffuse infiltration of the walls by polymorphonuclears and mononuclears with an infrequent eosinophile, intact mucous glands, a moderate hypertrophy of the muscle layer, occasional marrow reaction in cartilage surrounding bronchi, fibrous connective tissue replacement of the lumina of some of the small sized bronchi.

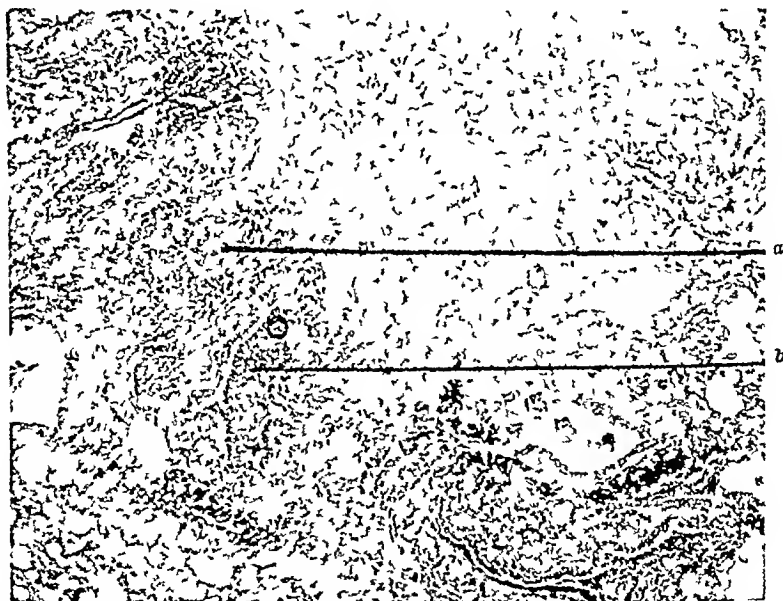


Fig. 10—*Bacterial asthma*. Cross section of lung and bronchi 1 mm or less inside diameter. The bronchi are distended and filled with an exudate. *a*, Atelectatic lung tissue. *b*, bronchus filled with a cellular exudate. (x30)



Fig 11—*Bacterial asthma*. Section of part of bronchus taken from the area marked with circle in Fig 10 a Muscle layer infiltrated by broken and intact polymorphonuclears and mononuclears the muscle bundles are moderately hypertrophic b lumen of bronchus filled by a cellular exudate consisting of broken and intact polymorphonuclears and mononuclears this field does not show a single eosinophile c lining epithelial cells (x500)



Fig 1 —*Bacterial asthma*. Section of part of wall of a bronchus mm inside diameter showing mucous glands which are diffusely infiltrated by cells (x60)

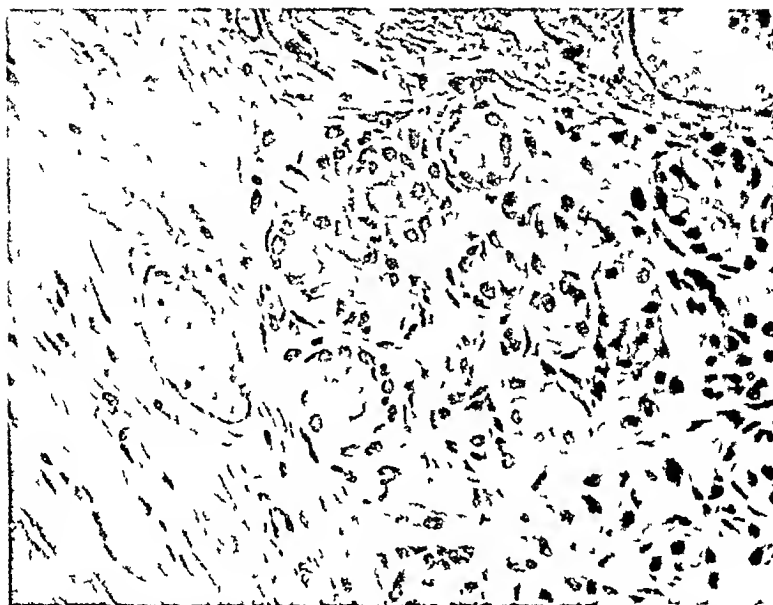


Fig 13—*Bacterial asthma* Section of part of bronchial wall showing several mucous acini. The nuclei are preserved the cytoplasm is intact and there is absence of mucous secretion

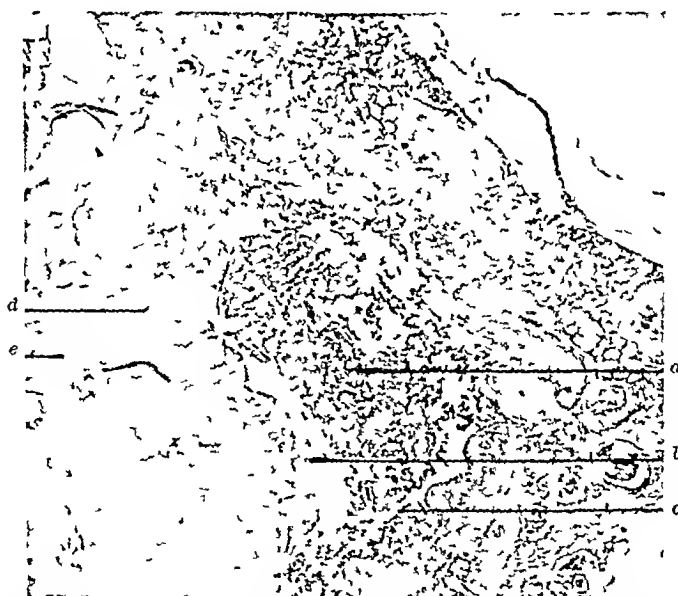


Fig 14—*Bacterial asthma* Cross section of bronchus with an inside diameter of 2 mm. a, Lumen filled with a cellular exudate b, apparent metaplasia of lining epithelium c, columnar epithelium on the opposite side of the transitional epithelium d, hypertrophic muscle layer e, intact mucous glands ($\times 60$)

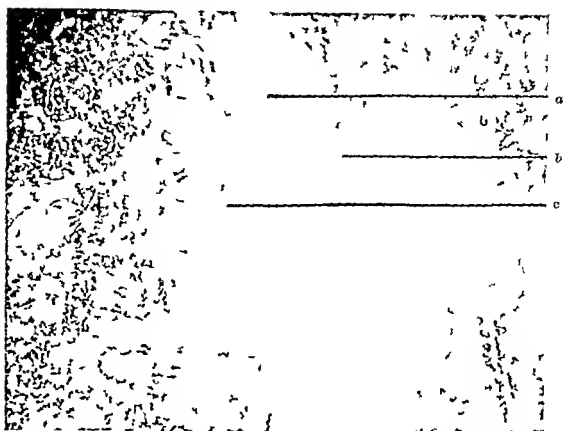


Fig 15—*Bacterial asthma* Section of lining mucosal cells of bronchu in Fig 14 a Lumen of bronchus b columnar epithelium c transitional epithelium (x300)



Fig 16—*Bacterial asthma* Section of bronchus a Cartilage showing bone marrow reactions (x100)

Pathologic Diagnosis—Chronic with superimposed acute purulent bronchitis, atelectasis of lung, subpericardial hemorrhages fibrosis of myocardium, slight, chronic with superimposed acute glomerulonephritis hemorrhagic necrosis of liver, acute arteritis of vessels of brain with encephalomalacia, early



Fig 17—*Bacterial asthma*. Section of lung showing atelectatic areas *a*, Area of atelectasis (x10)

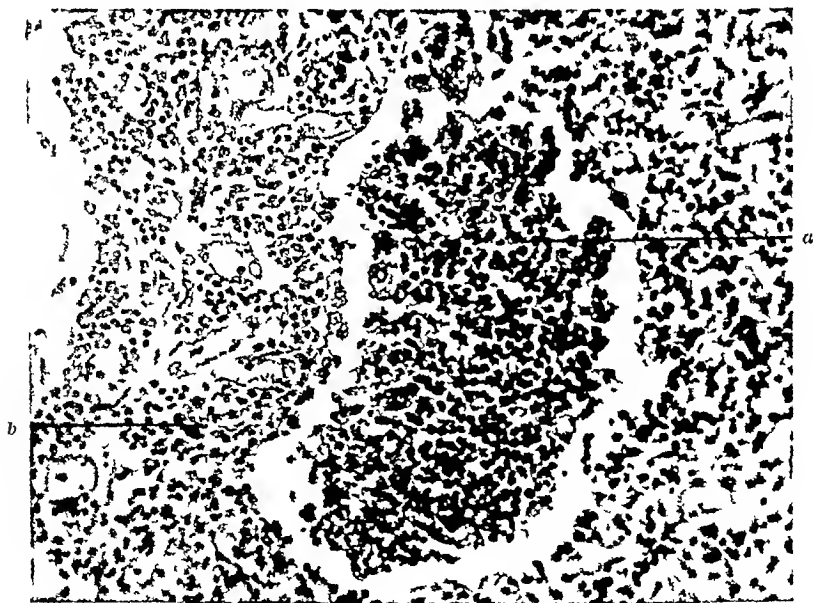


Fig 18—*Bacterial asthma*. Section of bronchus 0.4 mm inside diameter *a*, Lumen shows a cellular exudate consisting of broken and intact polymorphonuclears and mononuclears *b* the bronchial wall is diffusely infiltrated by mononuclears and polymorphonuclears the capillaries are distended (x300)



Fig 19—*Bacterial asthma*. Section of small bronchus. *a* Remnant of lining epithelium. *b* lumen completely obliterated by fibrous connective tissue which contains many mononuclear cells (x60)



Fig 20—*Bacterial asthma*. Section of brain showing blood vessels. *a* Blood vessel showing a diffuse infiltration of the wall by polymorphonuclears and mononuclears (x300)

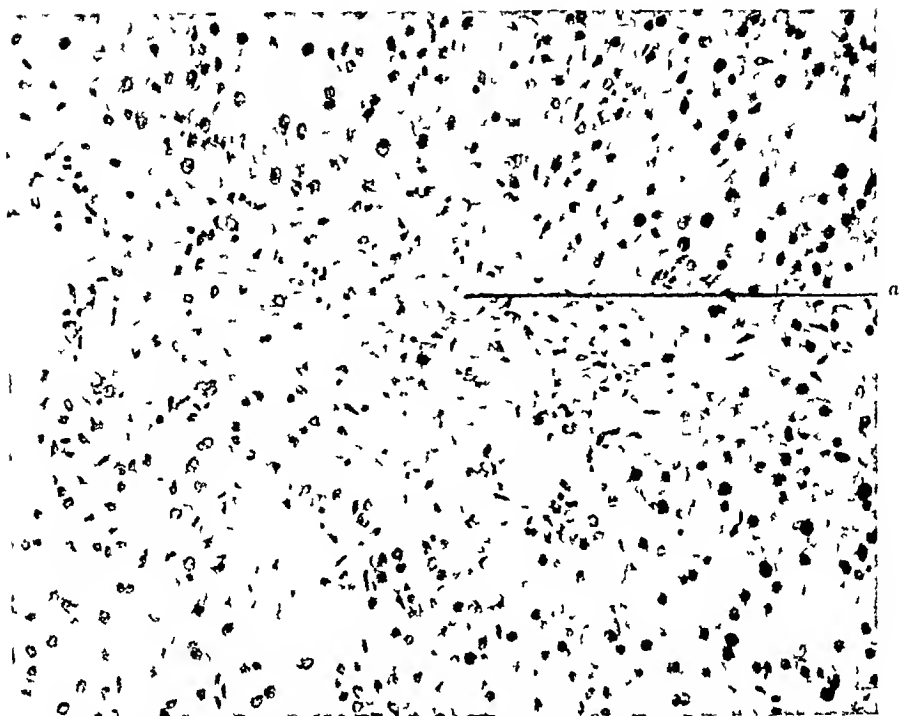


Fig 21—*Bacterial asthma*. Section of liver a Central zone of necrosis showing destruction of nuclei and cell cytoplasm and presence of red blood cells ($\times 500$)

PATHOLOGIC CHANGES IN NONBACTERIAL ALLERGIC AND BACTERIAL ASTHMA

Nonbacterial Allergic Asthma

- 1 Marked emphysema
- 2 No atelectasis
- 3 Lumen of large, medium and small bronchi and bronchioles partly or entirely occluded
- 4 Material in the bronchial lumen mainly mucoid which adheres to lining cells
- 5 Little or no destruction of the lining bronchial epithelium
- 6 Marked hypertrophy of the bronchial musculature
- 7 A large number of eosinophiles in the cellular exudate within the lumen and in the bronchial wall
- 8 Hypertrophy, hyperplasia and hypersecretory activity of the mucous glands

Bacterial Asthma

- 1 Slight emphysema
- 2 Moderate atelectasis
- 3 Lumen of small bronchi and bronchioles partly or completely occluded
- 4 Material in the bronchial lumina mostly cellular and it does not adhere to lining cells
- 5 Moderate to marked destruction of the lining bronchial epithelium
- 6 Moderate hypertrophy of the bronchial epithelium
- 7 Few eosinophiles in the cellular exudate within the lumen and in the bronchial wall
- 8 Hyperplasia of the mucous glands but little secretory activity

DISCUSSION

Patients affected by nonbacterial allergic asthma over a long period of time, not infrequently show a superimposed bacterial process. Such an infection confuses the pathologic picture of the original condition by altering

or adding new abnormal changes. Our case, both clinically and pathologically, shows absence of any secondary process. We are, therefore, dealing with an uncomplicated allergic asthma which eventually produced death of the patient. By the same token, the morbid anatomy is presumably the result of allergic asthma.

The anatomic changes in allergic asthma are apparently entirely restricted to the lungs. There is emphysema. The cause of the emphysema is still disputed, and the solution of this problem may advance greatly our knowledge of the etiology of asthma. It is generally accepted that emphysema is the result of occlusion of bronchial lumina. Although foreign material in form of mucous plugs or cellular exudates is consistently found within the bronchial tubes, many students of the subject hypothecate that the closure of the tubes is due to repeated spasmodic contractions of the bronchial musculature. There is a great deal of indirect experimental evidence that substantiates this theory. Hoover pointed out that stenosis of the bronchial tubes may be due to an evanescent edema of the bronchial wall. The bronchi show marked changes. The lumina of all except the largest bronchi are completely or partly occluded by mucous plugs. The failure of the patient to raise any bronchial secretion may be explained by the adhesion of the mucus to the bronchial epithelium. It is probable that when the bronchial lumen is completely filled and the further secretion of the glands is dammed, degenerative changes that are found in the mucous glands result. Whether the glandular hypersecretory activity is due to irritation from the cellular infiltration, or nervous stimulation, or is a result of muscular contractions remains a problem to be solved. The hypertrophy of the muscle layer has been a constant finding. In our case it is apparent. It has been assumed as a result of indirect experimental evidence that the muscular hypertrophy is due to spasmodic contractions. However, the bronchi may be contracting to expel the foreign material in the lumen and the hypertrophy is merely a result of increased effort. We have an analogy in the heart which hypertrophies to compensate for an additional load.

The bronchial wall shows edema and cellular infiltration. Many of the cells are eosinophilic. The cause of the presence of the cells and especially of the eosinophiles has been the subject of much discussion. Huber and Koessler review extensively the underlying basis for this cellular phenomenon and they conclude with Schlecht¹ that the eosinophilia is probably a protective reaction on the part of the body against decomposition protein products that are responsible for the allergic condition.

The pathologic changes in our case of bacterial asthma differ from those in the nonbacterial allergic asthma. The lungs in the case of the bacterial asthma show evidences of a chronic bronchitis with a superimposed acute bronchitis. The diffuse infiltration of the bronchial walls with loss of epithelial structure, the presence of bacterial emboli in the vessels of the brain, the acute nephritis, and the central hemorrhagic necrosis of the liver point to the entrance of bacteria into the blood stream. It may be assumed that in previous attacks of bronchitis a similar absorption of smaller amounts of

bacteria or their products occurred. Thus, bacterial sensitization, if such a phenomenon occurs, can be easily conceived in this case.

SUMMARY

The clinical histories and the morbid anatomy of two cases of asthma are presented. One case is of an uncomplicated nonbacterial allergic asthma dying during a paroxysm, the other is that of a bacterial asthma. The pathologic changes of the nonbacterial allergic type are differentiated from those of bacterial asthma.

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DISCUSSION

Dr Harry L. Huber, Chicago, Ill—First, I want to congratulate Dr Steinberg on the patience to take the time to study out these things, as I know, from the work I have done, how long it has taken him to do all this work. All these slides look very familiar to me.

I have another case to report which demonstrates one thing that we have not been able to find in other cases. Burkhardt reported finding a small ulcer in one of the bronchi. In explaining the bacterial type of asthma, or the asthma due to bronchitis, we have often wondered if an exhaustive search were made if we would be able to find some ulceration where it was evident that toxic materials were being absorbed. In this patient I am now working on, this ulceration is very clearly demonstrated. This was a woman physician in Chicago who had had asthma and had negative skin tests. She administered morphine herself and died following an overdose. The autopsy was done and in several different parts of the lung very definite minute bronchial ulcers were found. Although we made an exhaustive study of other cases reported we were not able to find ulcers. The presence of eosinophiles we have always thought was more indicative of the allergic type of asthma.

The mucous glands shown here are large. One point I wish to emphasize is the importance of the ampullae of gland ducts. These ampullae are shown to increase the thickness of the wall, or rather to decrease the size of the lumen, and they are probably responsible for the adherence of the mucus in the bronchi. The mucous glands secrete a very sticky mucus which pours into the lumen for a time, then as the bronchi contract, the muscle bands, being inside the mucous glands, pinch down on the ducts. The gland continues to secrete and large bags of mucus accumulate outside the muscle layer. This pushes against the mucous membrane and decreases the size of the lumen. The administration of adrenalin in these patients will very frequently, when relief begins to come, cause copious discharge of mucus. It is supposed these accumulations of mucus are being expelled at this time, and as these ampullae decrease in size, the size of the bronchus increases, and breathing is more easily done.

To distinguish between the allergic and the bacterial causes of asthma, I think it will be necessary to have a little more work done as it may be that the bacterial type will also prove to be allergic.

At one of the recent meetings in Washington it was suggested that arthritis and a number of other diseases that are now considered as infectious diseases, may be due to allergy. No doubt more extensive studies are now going to be made to link up a lot of the unexplained things which, if we have a clear understanding of allergy may help us a great deal.

Dr Milton B Cohen Cleveland Ohio—Recently I had the good fortune to observe one of these cases at autopsy. The patient was a woman about thirty seven or thirty eight who had a moderately developed mitral stenosis which was known to have existed for a number of years before she developed chronic bronchial asthma. This woman had been studied by cardiologists as well as allergists and it was the opinion of every one that the asthma was a true asthma, and had nothing to do with cardiac pathology. At no time had she had indications of decompensation. The heart disease was extraneous as far as asthma was concerned.

This woman was a patient who was not skin sensitive to any materials to which we tested her, but whose attacks could be very well controlled by environmental change.

We have at the city hospital at Cleveland, two particle free rooms. When I say "particle-free," I mean we have ninety nine per cent plus particle free air. A residence of ten days in this room afforded this patient relief and she required no epinephrin and had no attacks whereas previously she had attacks every day. She had gone home from the hospital feeling quite well and had been home about a week and was suddenly seized with a severe attack. She was brought into the hospital and within twenty hours died of respiratory failure, simply choked to death in spite of all the epinephrin and everything else that could be injected into her, or everything that we thought of doing. At no time did she have any evidences of cardiac failure.

At autopsy, the lungs showed a picture quite like the first case that Dr Steinberg described except that one pathologist said the bronchial muscle looked thicker another said it wasn't particularly thick but showed a mild bronchiolitis but in the gross the moderate sized bronchi were full of sticky mucoid discharge which could be pulled out, and came off more or less as a cast of the smaller bronchi.

There are one or two points of interest about bacterial asthma which I just picked up recently. Dr Wherry in Cincinnati who is in my judgment at least one of the best bacteriologists in the country has been studying many of these cases very intensively. It was surprising to me how many different organisms which could be got out of nasal or bronchial secretions were overlooked by bacteriologic methods. It was surprising he did not find some organisms which other men have reported to be present. However he has been able to demonstrate by the use of fifteen or twenty of these antigens autogenously prepared, definite tuberculin like reactions in many cases and has been able in quite a number to produce negative skin tests after rather prolonged courses of injections not just one negative skin test, because we know these things vary but skin tests remaining negative over considerable periods of time. He feels he is getting very nice results in such cases studied bacteriologically, as he studied them.

I noticed in the chart a reaction to tuna fish. Recently I made a survey of some hundred proteins that you buy on the market to find out how many had non-specific protein split products. One particular sample of tuna fish gave us a beautiful reaction after dialysis. What we did was to put this tuna fish in solution and dialyze against the same solution, and the tuna fish gave a reaction which we took to be due to histamine or some similar substance.

It wasn't worked out any farther than that.

Dr Ray W. Matson Portland Oregon—We have been interested in the doctor's talk on pathology in the finding of the eosinophilia. In a certain group of cases we have worked out in Portland Oregon we have found quite a series of sinusitis. One of the men there Dr Kistner, has not done as Dr Sewall has done in San Francisco. The radical sinus opera-

tion gives us some very valuable material. The finding of eosinophilia was also common in these sinus cases. Unfortunately, the bronchial asthma which was generally present in these cases was complicated by an infection, chronic sinusitis. The history, however, was clear in each case as to whether there had been an allergic history. The allergy had been worked out carefully by the cutaneous tests. The pathology in these chronic sinus cases was a hyperplasia of the columnar and squamous epithelium with often a mucous hyperplasia consisting sometimes of actual goblet cell formation. There was an edema of the submucous tissue, often extreme infiltration of cells consisting first of all of numbers of plasma cells. The next important cell was the eosinophile which sometimes infiltrated so quickly the whole field was pink with them. There were other types of cells in smaller numbers including the ordinary polymorphonuclear. Unfortunately, our figures do not indicate clearly the history of allergy or the history of nonallergy. The eosinophilia as reported by some workers is present in allergy and absent in the nonallergic cases.

We have had some extreme infiltration with eosinophiles in cases which have been carefully worked out by cutaneous tests and found negative to the ordinary allergens, and we have found the reverse. I just wish to report that, hoping that some of the other members would be able to compare this finding with their own.

Dr. Bernhard Steinberg, Toledo, Ohio—We were extremely fortunate not to find either clinically or pathologically any complication. The patient apparently had only the nonbacterial allergic asthma from which she died. We do not want to convey the impression that our labeling the second case that of bacterial asthma presupposes an absence of an allergic phenomenon. We are not concerned here whether there is or is not an allergic basis in those patients that begin with a bacterial infection and end with asthmatic attacks. It is, however, apparent that the pathologic changes are distinct in the bacterial type from that of the nonbacterial allergic. We are not generalizing, we cannot from two cases we are merely presenting the pathologic changes of two asthma cases. Only the repeated reports of additional cases may justify us in establishing a pathologic entity for the conditions under discussion.

ALLERGIC BRONCHITIS*

BY GEO L WALBOTT MD DETROIT, MICH

IN TICE'S *Practice of Medicine* Coole¹ describes a peculiar cough which he often found associated with allergic chorvza. The cough is violent and paroxysmal in character and is frequently accompanied by vomiting. He states that it is difficult to differentiate this cough from pertussis and believes that it is due to hypersensitiveness that has extended to the pharynx.

In no other monograph on allergy have I found reference to this type of bronchitis with the exception of that of Duke who states that many cases of allergic bronchitis are often misdiagnosed as tuberculosis, chronic bronchitis and bronchiectasis.

In a study on asthma in children A. Brown² asserts that a spasmodic bronchitis sometimes inaugurates asthmatic paroxysms in allergic individuals. This bronchitis is characterized by its abrupt onset as well as by its sudden subsidence. He believes that in children this bronchitis occurs more frequently than true bronchial asthma. Rowe,³ in speaking of allergic manifestations in children, states that a dry, irritating cough is a symptom of sensitization of the trachea and the bronchi and points out the importance of recognizing these atypical cases of asthma. Kahn⁴ expresses the same opinion.

In his series of cases, Peshkin⁵ found that among the factors that brought on the first attack of asthma, pertussis ranked much higher than measles. Considering the fact that measles is generally much more frequent than pertussis, he remarks upon this unexpected observation. In view of the similarity of allergic bronchitis to pertussis, one is inclined to believe that some of these patients with the history of pertussis might in reality have had allergic bronchitis, but it was not recognized as such.

TERMINOLOGY

The lack of an uniform terminology for the various coughs observed in connection with asthma has somewhat hampered the investigation of allergic cough. The term "asthmatic bronchitis" has been employed by most writers for any cough accompanied by wheezing and dyspnea, whether allergic or not. Others (Rackemann⁷) seem to restrict this term to the emphysematous bronchitis which frequently follows asthma of long standing. One well known author calls asthmatic bronchitis that type of asthma like cough in which protein sensitivity is not found.

Peshkin purposely avoids using the term asthmatic bronchitis because of its inadequacy. He introduced the term 'para asthma' indicating nonhypersensitiveness in contradistinction to 'asthmatic' or "hypersensitive bronchitis". However, the typical spasmodic cough described before, has not been elucidated by any of these authors.

*Read before the American Association for the Study of Allergy, Minneapolis, Minn. June 11, 1935.

CLASSIFICATION OF ASTHMATIC BRONCHITIS

The following is a classification of bronchitis occurring in asthmatics which I have found useful

- 1 Para-asthmatic bronchitis
- 2 Asthmatic bronchitis

The prefix "para" indicates that this group is definitely apart from hypersensitiveness. In making the distinction between hypersensitive and nonhypersensitive bronchitis the term allergic is not used because, in my opinion, it is desirable to restrict it to the true allergic bronchitis which will be described later. Some of the factors governing the distinction between asthmatic and para-asthmatic bronchitis are allergic history, heredity, skin sensitization, eosinophilia and response to epinephrin and related drugs. Among 163 patients observed with an asthma-like cough, only 109 were thus identified as true asthmatic bronchitis. Fifty-four had para-asthmatic bronchitis on the basis of such conditions as pertussis, chronic nonspecific bronchitis, tuberculosis, bronchiectasis, cardiac asthma and foreign bodies in the lungs.

Asthmatic bronchitis itself can be subdivided into three distinct types

- A Allergic bronchitis *
- B Intercurrent infectious bronchitis
(the common cold of the asthmatic)
- C Postasthmatic bronchitis

ALLERGIC BRONCHITIS

Allergic bronchitis can be considered a disease entity just as any other of the allergic diseases. It is characterized by the following features: sudden onset, spasmodic, dry cough, brought on by a sensitizing substance.

If sputum is obtained, it shows many eosinophil cells and few, if any, leucocytes. The cough is relieved by epinephrin or ephedrin. The x-ray picture shows the hilum glands considerably enlarged.

In a number of cases allergic bronchitis made its appearance before the onset of asthmatic attacks. I have also observed this type of bronchitis following x-ray treatment, especially in cases which responded favorably to this measure. Moreover, it occurred following administration of other therapeutic measures such as pollen or dust extract injections. There were two cases in which allergic bronchitis preceded the onset of asthma by a period of several years.

The following are some case reports

CASE 1—Pl., a two and a half year-old girl, was seen at home on account of "whooping cough." This was first noticed two weeks after birth when her mother discontinued nursing her. Lately, the cough had been frequently associated with sneezing and watery discharge from the nose. The child had had eczema. The father is asthmatic. A few months ago she was troubled with violent vomiting after drinking milk. Among the noteworthy findings there was a slight dermatographia, evidence of chronic tonsillitis and chronic sinusitis. The chest revealed a number of bronchitic bruits in the tracheal and hilum regions, and slight hyperresonance. There was an eosinophilia of 4 per cent and a 2 plus skin reaction to cow's milk. One-half cc of epinephrin immediately relieved the cough.

and diminished appreciably the degree of bronchitis. Upon eliminating cow's milk from her diet the child improved considerably.

CASE 2—Way, a girl 20 years old presented a similar condition which developed after several ragweed injections for hay fever. This cough was dry occasionally associated with vomiting. An x-ray picture revealed marked prominence of the hilar density. Sputum could not be obtained for examination. This cough stopped suddenly with her recovery from hay fever.

CASE 3—Ker, a ten-year-old girl had asthma of unusual severity for seven years. Because of her failure to respond to a number of other measures she was given x-ray treatments over the spleen. About four days after the third x-ray exposure she developed an unusually severe convulsive cough which might have been diagnosed as pertussis had it not been for the fact that the sputum contained a large number of eosinophil cells. The cough responded well to ephinephrin. After three weeks' duration the bronchitis subsided suddenly. The child has been under my observation for nearly two years. Since that time the cough has recurred twice, but she has never had another attack of asthma.

CASE 4—Smi, a four-year-old boy, had a history of frequent severe cough which has often been associated with vomiting. The mother observed that the cough was invariably evoked by the odor of cabbage. Skin tests revealed two plus sensitiveness to cabbage, one plus to carrots, chicken and veal. The boy's cough was temporarily relieved by avoiding these foods. However, during the following year he developed true asthmatic attacks the cause of which could not be discovered.

In addition to these, I have observed five other cases which can be classed in this group.

INTERCURRENT BRONCHITIS IN THE ALLERGIC INDIVIDUAL

Aside from the true allergic bronchitis just described there is another type of cough which may inaugurate asthmatic paroxysms. This type may be considered as the common cold of the asthmatic and is of rather frequent occurrence. In my series of 109 patients 63 presented a definite history of having bronchitis for a short time before the occurrence of asthmatic paroxysms. It is often very difficult to draw a separating line between these infectious colds and the true allergic bronchitis because only a very few of these patients feel sick enough to present themselves for clinical studies. However, the following observations could be made on this condition. Upper respiratory tract pathology such as sinusitis, nasal septum deviation and tonsillitis was usually the source of the cough. The sputum showed a large number of bacteria of the type usually present in the upper respiratory tract. Microscopically, we found many leucocytes but rarely eosinophils. Adrenalin was of no avail, whereas the ordinary expectorant cough mixtures were useful. The patients were benefited by autogenous vaccines and by the correction of nasal pathology. Occasionally positive skin reactions for bacteria were noticed but this was not a constant occurrence. The following is the report of a typical case sensitive to food, whose attacks were always initiated by respiratory infections.

CASE 5—Car, a twelve-year-old boy, had been afflicted with asthma since the age of three. The first attack started following pneumonia. Since the onset of the disease he had always been free from asthma during the summer months. In fall and winter the attacks occurred as often as once every two or three weeks. He had never had an asthmatic paroxysm without having previously suffered from an upper respiratory infection. The examination revealed a marked deviation of the nasal septum and chronic sinusitis and

the lung findings typical of asthma. He was sensitive to coffee, beans and rye. The skin tests for bacteria were negative. During the winter of 1927-28 he had only one slight attack after having received a series of autogenous vaccine injections.

An account of further instances does not seem to be indicated since the occurrence of these cases is quite common.

POSTASTHMATIC BRONCHITIS

This is the type that is often falsely referred to as asthmatic bronchitis. During the chronic asthmatic state we encounter a more or less continuous cough which finally produces permanent changes in the thorax and in the lungs such as bronchiectasis and emphysema. Being invariably the result of asthma the term postasthmatic bronchitis appears to be significant. Examples of this group were seen in 15 per cent of our series. The sputum contains thick pus, sometimes Charcot-Leyden crystals and also eosinophil cells. This condition is very refractory to treatment. Epinephrin is of no value. Frequently the chronic myocardial changes are the main indication for treatment. Bronchoscopic treatment accomplishing the dilatation of strictures of the bronchial tree and the elimination of large masses of sputum may be of value.

DISCUSSION

The main purpose of this paper is to draw attention to the relatively frequent occurrence of the true allergic type of bronchitis. It is of paramount importance to recognize it as such among similar conditions, because one might thus be enabled to detect bronchial asthma in its earliest manifestations. Moreover, by being aware of the similarity of allergic bronchitis to hylum tuberculosis and pertussis one might guard against the serious consequences arising from misdiagnosing these conditions.

The classification of asthmatic bronchitis into the three types is recommended mainly for therapeutic reasons. We have seen that removal of sensitizing substances, epinephrin and all the other measures which are applied for asthma are of great benefit in treating the first type, the true allergic bronchitis. For the second type, the intercurrent bronchitis of the asthmatic individual, vaccine treatment is indicated because these "colds" are of infectious character. Hygienic measures as usually advocated for the common cold and removal of chronic upper respiratory pathology might aid materially in the treatment. The type most refractory to treatment is the postasthmatic bronchitis in which permanent pathology of the lungs and heart is present.

SUMMARY

1. A certain type of bronchitis is described occurring in hypersensitive individuals which differs from the common infectious bronchitis. It is characterized by its sudden onset, by dry unproductive cough and by response to epinephrin and ephedrin. It is elicited by substances which are known to cause asthmatic attacks. This cough sometimes inaugurates asthmatic attacks and occasionally occurs before the subsidence of asthma and hay fever. In several individuals with inherited hypersensitiveness it was found as the only sign of their allergic constitution.

2 Aside from the occurrence of allergic bronchitis a great many asthmatics suffer from a bronchitis which clinically is distinct from true allergic bronchitis. This condition presents the features of the common cold and is termed "intercurrent bronchitis of the asthmatic."

3 A third type of bronchitis the postasthmatic bronchitis is described. It follows asthma of long duration and is often associated with permanent changes in the lungs due to allergic asthma.

4 In order to avoid ambiguity to further better knowledge concerning etiology and for the sake of a rational therapeutics the term asthmatic bronchitis should be restricted to the hypersensitive individual. Para asthmatic bronchitis is suggested for the nonsensitive asthma like cough.

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1056 MACCABEE BUILDING

DISCUSSION

Dr. Albert H. Rowe, Oakland, Calif.—I have been interested in this group of patients with allergic bronchitis for some time. I am very sure that the speaker is correct in stating that there is a definite type of bronchitis due to allergy. These patients may not show any asthmatic symptoms at all. I have in mind one little girl who came to me some three years ago. She was about nine years old at that time having had a cough all her life since birth. The parents had moved over various parts of the country to give relief to the child but without success. I did skin tests on the child and they were all negative. The child had a definite history of dislikes for certain foods. The mother had forced her to eat foods she disliked, which was very unwise.

By the use of "elimination diets" I found the child was sensitive to both wheat and milk. The child's cough stopped within four or five days after she was taken off wheat and milk. She got along very well for about six or seven months and at that time the mother thought she was so well she could return to the use of wheat and milk and when she did the attack returned and again was promptly stopped by the elimination of the foods. During that month's interval the child had been under the care of another physician who treated her from the point of view of bronchitis with steadily increasing trouble instead of improvement.

That is just one of the many experiences I have had in regard to this so-called allergic bronchitis. I think it is a term which is quite important to put into the literature and for us as allergists to recognize a type of cough bronchitis which is due to definite allergy, without asthmatic symptoms.

Dr. Alexander Sterling, Philadelphia, Pa.—May I ask how many of these series were tested and how many were sensitive or gave allergic skin reaction?

Dr. M. Murray Peshkin, New York City.—I am very sorry to have heard Dr. Waldbott employ the term 'para asthma' in his classification of asthma in a different sense than the original definition that I introduced in 1926. I plainly indicated then that the term

"para-asthma" was introduced as a substitute for the more objectionable term "obstructive asthma." It covers the type of asthma in which there exists a definite pathologic condition within the chest inducing tracheal or bronchial stenosis.

Before the subject of allergic bronchitis can be intelligently discussed, one must satisfy himself just what definition is to be applied to "asthma." Asthma is a clinical syndrome, quite readily diagnosed on physical examination of the patient. The diagnosis once made, all investigators of this disease proceed to search for the etiologic or exciting factors responsible for the presenting symptoms. Some authors would restrict the term asthma only to those patients exhibiting protein hypersensitiveness. Such a point of view is erroneous, since asthma, as far as children are concerned, is nothing more than a recurring dyspnea, more marked in expiration associated with wheezing, and the exciting cause may or may not be protein sensitization. Thus in the light of the present knowledge of allergy, the old definition of asthma is untenable since it only considers the attack as all-important. Physicians are learning more and more how to recognize diseases in their incipency. The early manifestations of asthma especially in children are definitely recognized as consisting only of recurrent spells of wheezing. To this incipient stage of asthma the diagnosis of acute bronchitis or asthmatic bronchitis is too frequently and erroneously applied. The attack itself is merely the final stage of the asthmatic state. A positive diagnosis of pure allergic bronchitis in children therefore can only be applied in those patients presenting symptoms of bronchitis in the absence of wheezing and in whom protein hypersensitiveness has been demonstrated.

Several years ago Dr. George G. Ornstein of New York called attention to a seasonal type of allergic bronchitis of pollen etiology. Dr. Waldbott's report is of interest because he has presented facts which are of extreme clinical importance. Allergic bronchitis may occur independently of the asthmatic state or it may occur during the intervals of freedom from symptoms of asthma. This condition is more readily demonstrated in children than it is in adults.

Dr. I. S. Kahn, San Antonio, Texas—I don't think we shall have to. I have on several occasions desensitized perennial hay fever in children with pollen. By unintentional overdoses of pollen extracts, I have produced this intense, annoying cough which is almost identical to pertussis, which dragged on ten days or two weeks unless adrenalin was used to check it up. In a community such as mine, where we have a good deal of pollen work particularly, with pollen hay fever and asthma, take a family of four children, and if you inquire into the family, you will find one child who is bothered with nasal trouble and a cough. I get a chance every now and then to prophesy such and such a child will develop into asthma and they usually do. I have seen this condition in adults, not by overdoses but actually in nature.

I had a case last year, a business man from New Orleans who came to San Antonio because of a chronic cough. The x-ray did not show anything. He had had hay fever which had stopped some ten or twelve years previously, and there had been no hay fever during that time. His skin tests were positive for pollens. I put that man in a pollen free room and in about five or six days that terrific annoying cough that had gone on for about ten years, was about gone. So we will, once in a while, run into these cases in adults as well as in children.

There seems to be an intermediate stage between hay fever and the actual bronchial asthma type, whether it is tracheitis or bronchitis.

Dr. George L. Waldbott, Detroit, Mich.—I am sorry that Dr. Peshkin seems to have discarded his term para asthma. Since there is such a widespread confusion on the subject of allergy due to inadequate terminology, I feel that this term is indicated. The prefix "para" means something aside from true allergic asthma. I thought it logical that any wheezing and dyspnea that is not allergic asthma should be termed para-asthma. I should have been interested in hearing this question discussed by some of the other men.

In regard to Dr. Sterling's question, I wish to say that I have had ten cases, among which three gave positive skin tests. However, in others the history definitely revealed

ensitiveness although the skin tests were negative. For instance one patient (J. K.) had severe allergic bronchitis whenever she ate cantaloupe.

One point which I should like to stress is the fact that I found marked enlargement of the lymph glands in most of the cases. Cooke, in his monograph in *Tee's Practice of Medicine* states that he observed enlargement of the tonsils in a patient showing the dry cough which I have termed allergic in my discussion. Since the tonsils are composed of the same tissue as the lymph glands one is inclined to think that enlargement of the lymphoid tissue may have some bearing on the causation of the cough.

INCIDENCE AND SIGNIFICANCE OF NEGATIVE SKIN TESTS IN POLLEN ASTHMA IN INFANTS AND YOUNG CHILDREN*

By I. S. KAHN, M.D., and EMMA M. GROTHALS, SAN ANTONIO, TEXAS

EVERY possible effort was made in this series of cases to eliminate all cases of nonpollen etiology. Cases giving positive skin tests to our other known specific etiologic antigens or clinically responding to nonspecific factors, as many of them did, were considered to be of additional pollen etiology only after the following conditions had been observed:

1. A definite history of seasonal and locational variations in the attacks.
2. Failure to secure results by the elimination of nonspecific and specific positive skin test factors other than pollen.
3. Relief of the asthma during a possible causal pollination season without the use of other measures than the pollen precautions we have described elsewhere.¹
4. Recurrence of the symptoms following the experimental relaxation of these precautions.
5. Induction of the usual cutaneous response and some nasal or bronchial allergic symptom indication by the subcutaneous pollen tests,² or by direct ocular or nasal application of pollen extracts or pollen corresponding to the history, with negative controls to other pollens.

The pollens employed were the usual of the eastern half of Texas: rag weeds, grasses, carelessweed, and mountain cedar. Testing was intradermally done with 1:50 extracts in Coe's³ or Stier's solutions,⁴ after preliminary testing with weaker dilutions. We have not had enough experience to state whether stronger extracts would reveal any differences in the figures to be quoted.

A wheal accompanied by pseudopodia, however small, was considered a definite positive skin test. Wheal and erythema formation, without pseudopodia, were considered questionable reactions.

In studying the tables to be presented due consideration must be given to the fact that my community is one of almost constant high atmospheric pollen concentration, where, as revealed by almost daily examination of pollen plates over a number of years, there exists never over a week in the entire year with

*Read before the American Association for the Study of Allergy, Minneapolis, Minn., June 11, 1918.

out the presence in the air in considerable abundance of some highly antigenic pollen. With our mild climate, and resultant constant outdoor, open window existence, with our low annual rainfall and frequent high winds, naturally pollen, as the sole or as a complicating factor in bronchial asthma, would be plausibly of high percentage frequency, and as a matter of fact does enter into the etiology of at least 90 per cent of the cases we see.

Pollen ceases to be a factor clinically, either temporarily or permanently as the case may be, in many of my cases who return to or visit more northerly communities of lessened pollen concentration and shorter seasonal duration. Whether any such percentage figure of negative skin tests as are here presented exists elsewhere, we are not prepared to say.

As seen from Table I,⁵ pollen asthma may start very early in life.

TABLE I
AGE OF SYMPTOM INCIDENCE IN 100 POLLEN ASTHMA CHILDREN

1 mo or under	1	In 5 of these cases hay fever symptoms existed from birth
1 to 6 mo	2	
6 to 12 mo	7	
1 to 2 yr	22	
2 to 3 yr	17	
3 to 4 yr	17	
4 to 6 yr	15	
6 to 8 yr	8	
8 to 10 yr	7	
10 to 12 yr	4	

A definite history of heredity was secured in 80 per cent of these cases. If we take into consideration the fact that pollen asthma in children is usually preceded by months or years of vasomotor rhinitis and recurrent bronchitis, febrile or otherwise, and that in 48 per cent of this series of cases, asthma started before the end of the third year, then our only possible conclusion is that pollen hypersensitiveness in many instances approaches the point of being natal or even suspiciously prenatal.

The percentage of positive skin tests according to the age of the child when first seen is of no value as such testing might have been performed after a history of asthma varying from a few days to the age of the child. However, in studying this relationship to the duration of the asthma, as shown by Tables II and III, a very interesting state of affairs is revealed.

It will be noticed at once that there is a sharp difference in the percentage of positive and negative skin tests in Tables II and III. In the children in whom asthma had recurred for a period of seven years or more, the percentage of questionable or negative skin tests was negligible, while in children whose asthma had lasted six years or less, the percentage reached the respectable figure of 40 per cent, and in infants and children up to two years of age equalled the number of positive tests secured.

The idea that the intracutaneous test with serial extract dilutions is a reasonably satisfactory measure for determining the degree of pollen hypersensitiveness existing in a given individual has been rather generally adopted by allergy workers. Accepting this idea, which on the whole and with rare exceptions that need not be referred to in this connection, as correct, it will

TABLE II

RELATION OF POSITIVE CAUSAL POLLEN SKIN TESTS TO DURATION OF ASTHMA IN 88 CASES

	POSITIVE	QUESTIONABLE	NEGATIVE
1 mo or less	1	1	0
3 mo or less	1	0	0
4 mo	1	1	0
6 mo	1	1	1
9 mo	2	0	2
1 yr	5	4	2
2 yr	2	2	2
3 yr	7	3	3
4 yr	7	0	1
5 yr	5	—	1
6 yr	7	0	4
Total 6 years or less	39	14	16

TABLE III

RELATION OF POSITIVE CAUSAL POLLEN SKIN TESTS TO DURATION OF ASTHMA IN 88 CASES

	POSITIVE	QUESTIONABLE	NEGATIVE
7 yr	10	1	0
8 yr	3	0	0
9 yr	1	0	0
10 yr	1	0	0
11 yr	1	1	0
12 yr	1	0	0
Total 7 yr or more	17	2	0

be noticed that, barring individual variations which must always occur in a statistical study of this kind the degree of sensitiveness increases in a heavy pollen environment directly in proportion to the duration of the disease. If this be true, then the negative skin tests secured in pollen asthma in infants and young children must be due not to any technical difficulties in testing or any differences in the reactive response between the skin of the young child and the adult, but to a degree of sensitiveness less than that possible for the skin to detect with our present methods. Such cases in the South in adults are not particularly uncommon.

If negative skin tests to 1:50 extracts in infants and young children are due in many instances to a low degree of hypersensitiveness as suggested with symptoms due to high pollen dosage, then by proper precautions against pollen overdosage alone, without the institution of other measures, the reception of atmospheric pollen might be held down to a point well inside this high tolerance limit, with either complete freedom from respiratory allergic symptoms or holding such symptoms correspondingly down to one or two light annual attacks following exposure to extremely heavy atmospheric pollen concentrations, yielding at once to ephedrin, and not worth the efforts or expense of specific desensitization. The practical methods of accomplishing this end have been described elsewhere.¹

We have at the present time 40 cases of early pollen asthma in children who have been handled with these pollen precautions alone without other measures for periods of from six months to two years. All have shown improvement, and in 75 per cent the results have been all or even exceeded what was aimed at. Several cases with typical positive skin tests to 1:50 extracts

are included in this series. In cases reacting positively to 1:500 extracts, we have feared to try this line of treatment, and proceed at once to usual desensitization methods. Fortunately infants and young children with early asthma, as has been seen, seldom fall in this latter class. One case with insufficient improvement was found much more sensitive by subcutaneous tests than what the intradermal results would have indicated, showing that this method of hypersensitivity determination by intradermal testing is not absolutely infallible.

In addition to the above, six children seen with symptoms for the first time during the 1927 ragweed pollination season, cleared at once and were kept perfectly clear throughout the entire season by this method of handling without medication of any kind.

For the last two years we have made it our business to ask of every adult patient under treatment for pollen hay fever or asthma, the number of children in the family and whether any showed symptoms possible of being construed as preasthmatic vasomotor rhinitis or bronchitis. A number of young children were thus found and the diagnosis of such preasthmatic state easily confirmed by the prompt and continued response to these antipollen precautions with complete immediate clearing of preexisting symptoms.

CONCLUSIONS

1 Negative skin tests are comparatively frequent in infants and young children suffering with bronchial asthma of pollen etiology.

2 These negative skin tests apparently indicate a low degree of hypersensitiveness.

3 Advantage can be taken of this low degree of sensitivity to secure successful clinical results by proper prophylactic measures without the institution of specific desensitization.

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MEDICAL ARTS BUILDING

DISCUSSION

Dr. Milton B. Cohen, Cleveland, Ohio—I am very much interested in this question of classification of hay fever by means of the strength of solution producing reaction, and I agree with Dr. Kahn that it cannot be done practically. I cannot classify cases as a, b, c, and d. Cases who give a definite reaction to pollen today may have a negative reaction on their skin tomorrow.

I have a case in point that I studied just two weeks ago. We do not see very many cases of pollen hay fever and asthma in our hospital as these are ambulatory cases. We were fortunate enough to have one admitted that I had studied some two or three years previously and knew to be sensitive to grass and ragweed pollen, and also to orris root. I had tested this patient one week previously to all three of these substances. I wished to demonstrate for the benefit of the interns who had not seen the reactions, so I made three scratches on the arm. To my chagrin, all of the skin tests were completely negative. We repeated the tests again on the next day and they were still negative.

We bled this patient on the day the skin tests were negative and passively transferred, to a patient who was normal. In the passively sensitized areas we demonstrated reaction to Timothy, Ragweed and Orris root. Thus the blood from a skin test negative patient proved to contain allergic antibodies. If such things occur how can we classify the severity of hay fever by the amount of pollen producing reaction?

I think very often we are inclined to be led astray by the fact that we feel that a positive skin test has definite clinical importance and we are led astray also when we feel that a negative skin test means that the individual is not sensitive to some particular substance. We must remember always we all make this same mistake and have to be constantly on guard against it, that every skin test is only a clue and it must be checked by clinical experimentation before we know what it means in any patient.

I think the method Dr Kahn talks of pollen precautions is a method of extreme importance, but I shall not discuss that in detail now because I shall talk about it tomorrow but a great many of the patients with hay fever who have mild hay fever can be relieved merely by proper pollen precautions without any need of preseasonal injections.

Dr Warren T. Vaughan, Richmond, Va.—Possibly similar types of cases exist in Virginia, but if they do I have not found them. I have, of course, seen negative food reactions.

The difference, presumably, is in the difference in pollen prevalence. We have a low pollen prevalence and we are more likely to get hay fever only in those who give good skin test reactions.

I feel that this paper emphasizes beautifully the value of these annual round table discussions in showing the great geographic difference in the etiology and the manner of treatment necessary in the allergic diseases.

Dr M. Murray Peshkin, New York City.—Dr Kahn's work has been of special interest to me because independently of each other we have been studying refractory sensitizations in children. In New York City the pollen concentration is considerably lower than that in San Antonio or Oklahoma City. Yet each year about 8 per cent of the children with asthma show negative pollen skin tests with positive histories of pollen sensitivity. A child is considered skin refractory to pollen when both the scratch and intradermal tests are negative. For the scratch tests pure pollen is employed and for the intradermal tests fluid extracts in concentrations of 1:50 and 1:20. A positive diagnosis is readily made by the ophthalmic tests which consists of placing pure pollen on the conjunctiva of the lower lid. In the residential sections of midtown New York the houses are tall and built closely together so that the avenues appear not unlike small canyons. Yet in spite of the low pollen count the patients who are skin refractory residing in such environment have at times severe pollen asthma. It is also of interest to note that in order to confer immunity on this type of patient a maximum dose of 1 cc. of a 1:50 extract of pollen must be administered.

Dr Kahn is to be congratulated for his intense and continued interest in this special phase of allergy because it forces to the realization that a test must also be developed that will aid in definitely diagnosing those cases whom are refractory to food and inhalant substances. At present such cases are labelled nonprotein sensitive. Pure pollen is soft and contains no chemical irritants thus making it suitable for conjunctival testing. Besides the pollen eye tests which react negatively serve most admirably as controls. Because of the nonspecific irritation induced by most of the food and inhalant powdered proteins these substances cannot be employed for eye tests.

Dr I S Kahn, San Antonio, Tex—This paper was a sort of an entering wedge to try to find out why negative skin tests occur. I have done most of my work with pollen because so many of my cases are pollen cases. A question comes up as Dr Peshkin says, if we can have pollen cases with negative skin tests, why cannot we have animal hair, feather and food cases with negative skin tests? Probably we do. Certainly I have seen a number of food cases, definitely clinically worked out as food cases with absolutely negative skin tests.

If we go on the bases in this asthma work that we are going to depend on skin tests alone to guide us for diagnosis, we are going to make mistake after mistake. For instance, that child which I showed on the record with positive skin tests to food, animal hair, and negative pollen tests, was a definite pollen case clinically. One of the reasons we make mistakes on our asthma cases today is the fact that we pay strict attention to our skin tests and nothing further. We go to work and say because a case is positive to feathers and negative to pollen, it must be a feather case. I did that too until I had some disasters and failures.

I do not know so much about northern cases as I do about those in the South, but I live in a health resort and cases float into San Antonio from all through the north, and you would be surprised at the number of negative pollen skin tests in chronic asthma cases that I see in San Antonio that originate in Chicago or any of the northern cities. The eye test of Dr Peshkin with crude pollen has proven extremely valuable in my work, and it seems specific. However, it is not an infallible test. In Texas, with the cases I see, it will clear up fifty per cent, that I have to find out by the subcutaneous or some other method of testing.

SOME CAUSES FOR FAILURE IN THE SPECIFIC TREATMENT OF ALLERGY*

BY WARREN T. VAUGHAN, M.D., RICHMOND, VA

A GUNN AULD¹ in the *British Medical Journal* for February 4, 1928, speaks of the so called specific treatment of asthma as follows: "It may be said that the method has largely broken down. Nevertheless a clear light has been thrown on the *modus operandi* of many of these substances and some excellent results have been obtained so that it is unjust to depreciate it. It is a distinct advance in our knowledge and the time may come when definitely specific cases can be made to respond more to specific therapy though at present a considerable number do not do so." Auld refers I take it not only to desensitizing injections but also to protein avoidance and to the value of skin tests in general. Men like Coke in England, and others on the Continent such as VanLeeuwen in Holland and Gianni in Italy, have become convinced of the value of specific methods of examination and therapy. But on the whole the continental attitude has been one of some indifference.

In this country on the contrary there are many of us who firmly believe that with this method of approach we have already passed the stage where we wonder whether the method will work in the individual case, and have reached a point where we are vexed and perplexed when it does not work and proceed at once to find the reason for its failure.

In this paper I offer a brief discussion and classification of some of the causes for therapeutic failure which I have encountered. At the same time I have not hesitated to draw freely on the experiences of others so that the discussion might be made more complete.

Robert Hutchinson² in his recent masterly discussion of the principles of general diagnosis, attributes poor results to (a) defective observation, (b) defective knowledge or (c) defective judgment. He says: "It is commonly said that more mistakes are made from not looking (defective observation) than from not knowing (ignorance). However this may be I believe that still more mistakes arise from bad weighing of evidence (lack of judgment)." These remarks may well be applied to the study of allergy. Following the same line of thought, I would offer four general reasons for therapeutic failure, namely superficiality, ignorance, poor judgment and insurmountable obstacles.

SUPERFICIALITY

The superficial thinker and investigator will meet with little success in the specific study of the allergic diseases. The successful allergist or immunologist or whatever you choose to call him must be the very impersonation of the question mark and in his travel with his patients along the road to

*Read before the American Association for the Study of Allergy, Minneapolis, Minn. June 11, 1928.

understanding must be willing to journey down many a seductive appearing by-path, chasing many a will-o'-the-wisp in order to be certain that no discovery of real significance will be overlooked. Fortunately there is usually no great hurry. The patient has had his disease for many years and is well content to allow ample time for thoroughness provided he has reasonable assurance of ultimate relief. Not only must the allergist be diligent, he must also possess a ready imagination. He must look upon all things in the immediate and remote environment of the patient as potential allergens even though they be so minute as to be invisible under the microscope or so apparently innocuous as mounted moose heads or chocolate coated pills.

The rôle of the allergist is that of a detective. His client has lost something, his health and sense of well-being. He is able to give some clues but it is up to the examiner to ransack the history of the past, even including the experiences of remote ancestors, and to make a most painstaking examination at the site of the loss and throughout the surrounding territory so that he may leave no clue untouched in his effort to return good health to his client. No clue is too insignificant to be carefully studied in orderly arrangement with the other clues.

IGNORANCE

Ignorance is quite a different attribute from superficiality. Neither is an insurmountable obstacle. I refer here not to the ignorance which accompanies lack of interest but to the ignorance caused by our lack of understanding of the fundamental processes of allergy. Twenty years ago our ignorance of the allergic aspects of asthma, hay fever, eczema and the other diseases may be said to have been complete and it is reasonable to assume that as time goes on our knowledge and understanding will be steadily enhanced. Ignorance may be properly subdivided into ignorance of allergy, that is, ignorance as to the basic processes involved, ignorance of allergens, that is, failure to recognize certain substances as potentially allergenic and failure to understand the exact nature of allergens, ignorance of technique which in the hands of the best is undoubtedly responsible for a percentage of failures, and ignorance of the patient, failure to realize that we must deal not only with the seed of the disease but also with the soil in which it falls and that each patient is constituted differently.

IGNORANCE OF ALLERGENS

Our ignorance of allergens is due in part to feeble curiosity and poor imagination and in part to our uncertainty as to the exact nature of allergens and what varieties of reagents this class may include. It requires a nimble thinker to demonstrate the allegenic rôle of such substances as grain dust, moulds, intestinal parasites, moths in feather pillows, sparrows nesting outside the bedroom window, hog bristles, and urease. Even with such rare causes the intelligent patient will often himself give the clue but frequently we are not alert enough as to the possibilities to follow it up.

Let me propose a tenet and be bold enough to call it an axiom. There is a specific allergenic cause for nearly every case of true allergy. Failure to

find the allergen does not demonstrate its absence. If we will doggedly persevere in our search for the specific cause after the usual possible causes have been ruled out, we will materially reduce the proportion of our failures.

A most stimulating experience along this line has been related to me by Dr. Earl D. Figley, of Toledo, Ohio. He tells of an asthmatic in whom he made a most exhaustive search for the etiologic factor even to the point of visiting the home and making extracts of a long list of substances in the house but without avail. Many months later Dr. Figley³ was investigating the possible cause for attacks of asthma in a group of individuals living in the neighborhood of a castor oil factory. He obtained some of the dust from the castor bean and most of the asthmatics in the neighborhood were found strongly sensitive thereto. Many other asthmatics living within a radius of one or two miles of the plant then came in for testing and among them Dr. Figley found his old friend. He was sensitive to the dust of the castor bean.

I have a horse asthmatic who is never near horses but who will develop an attack when her brother sits beside her at the dinner table, after a horse back ride. This is avoided when he changes his clothes prior to dining. Here we might borrow from the bacteriologist and speak of a carrier state. A third individual is responsible but the contact with horse epidermal protein is just as actual although more difficult to trace than it would be did the asthmatic work in a stable.

Balyeat⁶ whose botanic survey of Oklahoma has been most comprehensive has convincingly demonstrated the need of accurate knowledge of the local botanic flora in the patient's immediate environment. Even in the single state of Oklahoma the predominating etiologic pollen may be quite different in different counties. But before this could be known and applied laborious weeks were devoted to the study. Prior to his classification I dare say that some cases were considered unsuccessful because the right pollen was not available for testing.

The first battle cry of the allergist should be, 'The specific allergen is there, somewhere—find it! Where there are several find the right one.'

I have found the accompanying questionnaire of some help in finding the cause in the occasional obscure case and in establishing the probable allergic nature of the disease. The discussion of each question with the patient occasionally brings to the surface a suggestion of real value in our search for the cause. It is what we all do in a more or less routine manner but possesses the advantage of a somewhat orderly and quite inclusive classification.

IGNORANCE OF ALLERGY

Just when we have about concluded that we understand the nature of allergens some new evidence is put forward to again effectively spread the shadow of doubt. There is so much evidence that allergens are protein in nature that we become rather disconcerted when Grove and Coca⁴ suggest and are supported in their suggestion by Black⁵ that pollen and dust allergens are probably not nitrogenous. We have tried to explain drug idiosyncrasies in terms of the usual concept of protein allergy but the fact remains that these

cocaine application in the nose is quite real when it does occur and its action is probably upon the nerve endings. Does vagotonia or sympatheticotonia or better dystonia or imbalance between the two systems play a rôle irrespective of allergenic contact? There is much evidence that it does. Simple flight in an allergic individual may bring on an attack. Often we do not give enough attention to the so-called asthmatic habit. The occasional morphine addict, made so in his search for relief from asthma can stir up a real good attack with wheezing râles through the chest when he enters a new doctor's office to beg anew for the soothing poppy. One of my most resistant asthmatics tells me that after she has been able to do without adrenalin for three or four days, she tries to fight the next bad attack rather than use adrenalin, for she has discovered that if she can stick it out through the night the following night is likely to be better. If she does not, she usually requires adrenalin on the second night also.

Is mechanical irritation interfering with the results in a case of eczema? A woman with seborrhoeic eczema of the forehead, two plus sensitive to lobster, pyrethrum, pepper and one plus to lactalbumin, cocoa, rice, celery and strawberry and with a clearcut allergic history in that tomatoes cause urticaria, strawberries produce "ptomaine poisoning" and she has frequent attacks of vasomotor rhinitis, was placed on specific food avoidance without recognizable benefit to her eczema. The next question was is this a true eczema or is it a seborrhoeic dermatitis possibly dependent upon trichophyton infection. A soothing parasiticide ointment containing sulphur was prescribed. The eruption on the forehead cleared up promptly. Conclusion—diagnostic error! But no, as soon as she started in again with the ingestion of milk the rash returned as bad as ever. This disappeared again on milk avoidance but the ointment was continued.

A local physician tells me of a family with several asthmatic children the father of whom many years ago had had syphilitic infection. He assures me that he is able to keep them all asthma free by employing periodic mercury inunctions.

What of the infection factor? Whether or not we side with those who insist that one may be specifically sensitive to the protein of specific bacteria, it is a fact that one way or the other infection plays a part in upwards of 40 per cent of asthmatics. If we deny that infection plays a part we are left helpless in the treatment of too large a percentage of asthmatics. We need not take sides in the issue over specific bacterial sensitization unless we care to but the occasional spectacular relief after autogenous vaccine therapy compels us to consider infection while considering the patient as a whole.

POOR JUDGMENT

Fortunately experience will ripen judgment. One who has had any wide experience with hay fever is not likely to emulate the tyro who because a person is suffering from late summer and autumn hay fever tries ragweed desensitization in spite of the fact that the symptoms begin around August first. But allergy is a great field for speculation and most of us have our

The Vaughan Graham Clinic Richmond Va

Asthma and Hay Fever Detail

Name _____		Date _____	
Identification		Foods	
Asthma		Suspect	
Bronchitis		Symptoms	
Croup		Dislikes	
Pneumonia		Symptoms	
Hay Fever		Cravings	
Sneezing		Combinations	
Colds		Overeating	
Coughs		Previous diets	
Wheezing		Results	
Nasal obstruction		Climate	
Stiffness		Season	
Sinus trouble		Altitude	
Itching palate		Temperature	
Itching eyes		Humidity	
Itching nose		Wind direction	
Itching throat		Light	
Itching ears		Specific localities	
Itching larynx		Bedroom	
Lacrimation		Always same room	
Olfactory impairment		Attacks elsewhere	
Taste impairment		Sleep alone	
Diurnal variations		Room alone	
Associated conditions		Pillow	
Eczema		Mattress	
Urticaria		Covers	
Pruritus		Wall coverings	
Other eruptions		Rugs	
Headaches		Pets	
Migraine		Ventilation	
Food upsets		Plants	
Ivy poisoning		Home	
Urinary frequency		Describe house	
Asthma		Time living there	
Hypertension		Heating plant	
Colitis		Dust	
Epilepsy		Cleaning methods	
Family History		Suspect rooms	
Asthma		Pets	
Hay Fever		Mice	
Rhinitis		Flowers etc.	
Urticaria		Grounds	
Eczema		Flowers	
Skin disease		Weeds	
Headaches		Pets	
Migraine		Live stock	
Food upsets		Contents of barn	
Colitis		Neighborhood	
Ivy poisoning		Time living there	
Hypertension		Other cases	
Epilepsy		Their causes	
Contributing factors		Trees	
Chilling		Plants	
Electric fan		Fields	
Draughts		Weed	
Dust		Insects etc	
Smoke		Factories	
Tobacco		Smoke	
Flowers		Odors	
Face powder		Occupation	
Odors		Detail	
Perfumes		Contacts	
Soap		Ventilation	
Crowds		Dusts	
Theatres		Flowers	
Coryza		Miscellaneous	
Drugs		\ ray chest	
Animals		\ ray sinuses	
Pets		Other \ rays	
Furs		Gastric Analysis	
Bedding		Remedies tried and Results	
Catamenia			
Exhaustion			
Shock			
Specific localities			
Change of locality			
Constipation			
Nose and Throat Operations			
OPERATION	DATE	REASON	RESULT
1			

pet theories. Occasionally in our enthusiastic support of an as yet unproved theory we fall into the rut of following some special line of therapy in the hope of proving our point, and fail to observe that too large a proportion are not obtaining the relief which reason and experience have shown may be obtained by the more usual methods. No use to try to treat all asthmatics with tamponage of the ethmoidal sinuses when your better judgment will tell you that not all are infectious in origin and that an edematous nasal mucosa does not necessarily indicate local bacterial invasion. Why treat an asthmatic with extensive bronchitis and bronchiectasis only by specific protein avoidance or desensitization and hope for best results when you must know that appropriate nonspecific methods must be applied to the other local pathology for greatest relief?

INSURMOUNTABLE OBSTACLES

Insurmountable obstacles beyond any that might fall in the preceding categories will prevent best results in some cases. Pronounced pulmonary emphysema, advanced bronchiectasis and some cases of sinusitis will fall into this group. But good judgment and increasing knowledge will eventually reduce the total number of insurmountable obstacles.

NONCOOPERATION

Noncooperation on the part of the patient or the referring physician is sometimes a cause of failure. The better the patient understands the rationale of treatment the more willing he usually is to cooperate. Physicians who have not made a special study of asthma sometimes do not seem to understand complete avoidance or to appreciate its importance. All of us I am sure have had new patients assure us that they had completely avoided wheat ororris root or feather dust, only to discover on further questioning that they had unwittingly continued in daily contact.

CONCLUSIONS

The title selected for this paper is "Some Causes of Failure." While I have endeavored to make my remarks inclusive particularly as to classification, I make no claim to completeness. I presume that I should conclude this paper by offering an outline of successive steps to be performed in the study and treatment of the resistant and obscure case. Unfortunately, however, allergy cannot be treated by rule of thumb. Every case must be treated on its individual merits and in accordance with its own peculiarities. The detective who works merely by the book, no matter how well written, will often fail to solve the difficult case. My plea is twofold, first bear in mind that greatest promise lies in finding the specific allergenic factor and, second, remember that whether this is found or not, you are treating not a disease but a patient with a disease, not asthma but an asthmatic.

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707 MEDICAL APTS BLDG

FURTHER OBSERVATIONS ON THE USE OF FILTERED AIR IN THE DIAGNOSIS AND TREATMENT OF ALLERGIC CONDITIONS*

BY MILTON B COHEN MD, CLEVELAND OHIO

AT THE meeting of the American Association for the Study of Allergy held in Washington, D C last May I reported my observation on the effect of filtered pollen free air on six patients with seasonal hay fever and asthma. These patients were treated in their own homes by means of electrically operated mechanical filters which produced and maintained pollen free air in their bedrooms. The filters consist of a motor driven suction fan covered by a filter bag made of several layers of a specially woven woolen cloth covered by one layer of cotton cloth with the entire mechanism enclosed in a metal housing. The intake of the machine was connected to a special screen fitted to the window opening by means of a metal pipe (Fig 1). As pollen free air was forced into the room through the filter a slight positive pressure was soon produced and the old air rushed out through all the cracks and crevices. Thus it was unnecessary to seal the room or to change it in any way, and it became practical to treat patients by means of filtered air in their own homes. These filters remove 62 per cent of tobacco smoke with a particle size of 0.27 micron and 95 per cent of zinc oxide with a particle size of 0.304 micron. They can be depended upon to remove all noncorrosive particles larger than one micron. It is readily seen that almost all dust particles are removed also and that by means of these filters it is possible to produce and maintain dust free rooms. However for experimental purposes it was desirable to have filters of even greater efficiency than these and accordingly, machines were produced which were capable of delivering 250 cubic feet of air per minute of 99 plus per cent particle free air.

With filters of the standard and the experimental type, studies were made to determine the following points:

1. Are there variations in the severity of hay fever and its accompanying asthma due to the dosage of pollen breathed in?

From the Medical Service and the Allergy Clinic of Ml Sinai Hospital
Read before the American Association for the Study of Allergy Minneapolis Minn
June 1, 1928

2 Will the breathing of pure, pollen-free air for a small number of hours daily suffice to keep the daily dose below the patient's threshold of reaction and maintain him free of symptoms, or will permanent residence in pollen free air be necessary in (a) patients who have received no preseasonal pollen extract injections, and (b) in those who have failed to obtain complete relief by these means?

3 When symptoms are established and a patient is removed to an environment free from pollen, how long will it take for the acute symptoms to subside?

4 Can inhalant-free rooms be used to determine (a) whether symptoms are due to inhalant extrinsic causes and (b) as in the case of pollen, how long a period of residence in such a room is necessary for the control of symptoms, and (c) after the acute symptoms have subsided, how many hours' residence daily is required to prevent their recurrence?

For the study of pollen disease it was necessary only to install a filter in the patient's bedroom a few days before the beginning of the season of pollination of the plant to which he was sensitive, and to instruct him to sleep in this room. The usual occupation was continued, though golf, tennis, and auto rides were discouraged. As the season progressed, a report was made of the presence or absence of symptoms. Whenever the slightest symptoms arose, additional hours in filtered air were advised, until for each patient there was worked out a satisfactory schedule of time in filtered and in outside air. Quite early in these studies it was seen that the number of hours in filtered air necessary for any patient varied directly with the air pollen concentration. Eight hours usually sufficed for all patients during the first few days of the season. As the season advanced, more hours of residence were required, until the peak of the season, after which the requisite number of hours gradually diminished. For the mild patients, those who had previously been relieved by residence in average hay fever resorts or by a short course of preseasonal pollen antigen injections, from eight to twelve hours were sufficient. For the cases of moderate severity, from twelve to eighteen hours were required, and for the most severe ones, from eighteen to twenty-two hours were needed at the peak of the season to remain symptom-free. All patients who had received partial relief following preseasonal treatment were rendered symptom-free by a residence in filtered air for a few hours a day, usually not exceeding eight. In those patients in whom symptoms were well established, it required from two to seven days' residence in filtered air for the symptoms to subside. Then recurrence could be prevented by partial residence, as in those who began the use of filtered air before their symptoms began.

There are many asthmatics in whom it is extremely difficult to determine the etiology either by clinical experimentation alone or by skin tests followed by clinical experimentation. Usually, these are regarded as cases of bacterial asthma with its unsatisfactory treatment and poor prognosis. We have studied ten patients of this type, who have been placed in dust-free environments for periods of from seven to fourteen days. In creating a dust-free room the following procedures are followed. A bedroom of not exceeding 1500

cubic feet capacity is thoroughly cleaned the walls are washed or repainted and repapered, all draperies and rugs, etc., are removed, the floors are oiled or waxed, all furniture is removed except a bed and a chair or two, which are thoroughly scrubbed the mattress and pillows are covered with rubber sheeting or with Dupont's new satin fabricoid, which is to be preferred. If the home is heated by hot air the furnace pipe must be hermetically sealed. A filter is then installed taking air from an adjoining room through the opening in the wall. The clean room is then held full of clean air under pressure, and no leakage from the rest of the house occurs. Five of these ten patients lost their asthma within a week, and all have remained asthma free when similar environments have been produced at home and they have remained in dust free air at home for from twelve to fifteen hours a day. This is in agreement with the reports of W. Storm van Leeuwen who relieves a large percentage of all asthmatics by removing them to dust free rooms. His apparatus is, however, extremely cumbersome and very expensive.

While it is realized that ten cases are not sufficient from which to draw any conclusions it is obvious that about half of the patients in whom a diagnosis of bacterial allergy would have been made by exclusion, can be considered as inhalent asthmatics.

This report is presented in the hope that others may make similar studies so that more allergic patients may have adequate diagnosis and satisfactory relief.

10616 EUCLID AVENUE

DISCUSSION

Dr I. S. Kahn, San Antonio, Tex.—I think this work with filtered air is one of the valuable contributions we have had. I have been experimenting with pollen free rooms for probably three or four years, using first inside court rooms in a downtown hotel, then home made filters, and then Dr. Cohen came out with this apparatus.

These pollen free or pollen reduced rooms have brought up some very interesting points with regard to etiology in bronchial asthma. I have had the same experience that Dr. Cohen has had, finding that there is a vast percentage of previously considered bacterial asthmas with negative pollen skin tests which will clear up in such rooms.

When we had this discussion brought up in our meeting in Washington last year, Dr. Cohen had the idea that if the asthma case did not clear up in five days in a pollen free room it could not be a pollen case. I told him at that time he was wrong on that point, because I feel that it will take a considerable longer period of time before some cases will clear up. Hay fever cases clear promptly but such asthma cases who have taken adrenalin for years have so little resistance power and do not seem able to get rid of this pollen toxin to a tolerance point within three or four days.

The last case I had behind a Cohen Filter took twelve days to clear. I have seen a filter require from one to two months in a sick asthma case before final clearing and in one case in a pollen free room, the longest I have ever seen, kept steadily in bed took five months before it was absolutely clear—a sick adrenalin-exhausted individual.

I was glad to hear of the improvement in the Pollenair Company filter to get rid of the lint given off. I have put pollen plated out in the room behind the originally designed Cohen filter, and you would be surprised at the amount of hair particles trapped. Certainly some sick pollen cases are so annoyed by the lint and fuzz that comes off the machine that it prevents them from clearing up as they should. So simply because a case would not clear under the original Cohen filter, was not an indication that it was not a pollen case.

The apparatus Dr. Cohen has is a very pretty machine and I find it very, very satisfactory. However I have been using a home made machine for three or four years.

which is just as satisfactory, and any patient can build it for a few dollars. The machine I have works on the same principle except that I use an ordinary window screen and a backing stuffed with half the thickness of absorbent cotton such as comes in an ordinary pound roll, which is basted between cheesecloth, and an ordinary twelve or fourteen inch electric fan, and if necessary, two fans. The air currents from the electric fans will keep the pollen from coming in from the opposite direction or from the sides. In hot weather in Texas, we cannot use the patented filter because the room gets stuffy, smells, and I have to put in an additional electric fan or switch over to my homemade apparatus. I think any of you who are handling asthma cases are making a big mistake, both for diagnosis and therapeutic purposes, if you are not employing pollen filters of some kind in your work.

Dr M. Murray Peshkin, New York City—Dr. Cohen is to be complimented for encouraging the development and manufacture of the pollen filter. This machine has proved its efficacy by relieving patients with hay fever.

To my mind the pollen filter can also be employed for a purpose other than that it was originally intended. Patients whom are classed as environmental asthmatics and in whom all forms of specific and nonspecific therapy failed to give relief, have always been difficult to do anything for. In other words such a patient's tolerance for the particular environment is so low that appropriate therapy makes no impression on his regulatory mechanism. If he is removed from his environment, perhaps to the seashore or open country he frequently becomes entirely relieved from asthma. Upon returning to his former environment in spite of the fact that it has been rendered allergically proper, asthma recurs. The installation of an air filter in the bedroom of such a patient may be of extreme benefit.

Van Leeuwen has constructed a number of dust and miasma free chambers in which patients with asthma sleep. Some of these patients were able to go about their daily work, earning a livelihood, in extreme comfort while others were entirely relieved of asthma. The patients who were not greatly relieved were administered nonspecific treatment with extreme benefit. The cost of constructing these special chambers is very high and therefore beyond the reach of the average patient, whereas the installation of an air filter in a room properly prepared is comparatively inexpensive. The cost in fact is small when compared to the amount of money expended in an effort to obtain relief from the distressing symptoms of continuous asthma.

There is another type of patient with asthma who when removed to the public ward of a hospital, perhaps only a short distance from his home, becomes entirely free from symptoms within one to three days, and remains so during his entire stay in the hospital. This type of environmental asthma is obviously not expected to become free from asthma after the installation of an air filter in his home.

Dr. Milton B. Cohen, Cleveland, Ohio—With regard to the question of stuffiness in these rooms, it not only happens in Texas but it happens in Cleveland or anywhere else. You get exactly the same temperature in the room inside as out when you have absolutely quiet air. All one needs to do in this circumstance is put a fan in the room to create a circulation of the air so the patient feels moving air, and then the room feels comfortable.

I did not refer to van Leeuwen's work because I did not want to take time on literature. He has two types of rooms. They are of the same type, but he ventilates them in two ways. He employs a metal room, 6x6x9, which is hermetically sealed and which is ventilated by means of an ordinary suction fan and a pipe taking air from the height of thirty or forty meters. He takes ordinary, plain outside air, unfiltered, and passes it through this small chamber which is absolutely free of dust. All he does in such cases is to remove house dust because he has built within his house a dust free place and he uses outside air. He does state there is a small percentage of asthmatics, three, four, or five per cent, in whom the ordinary outside air borne things, he calls them miasms, produce trouble. In this type of case he has special clean air, not filtered, which he cleans by means of refrigeration. He claims if you freeze air, or bring it down to a low temperature where all the moisture comes out, it will bring out all the particulate matter and this air which remains

in the refrigeration apparatus is warmed and brought into the house, and it will be free of dust. He wrote me that the ordinary chamber cost \$400 in Holland and from the pictures of the special installation, it looked as though it would cost \$7000 \$8000 or \$10,000 to equip a house with such apparatus

The filters which are being sold are the ordinary filters which will handle sixty two per cent of tobacco smoke and all particles larger than half a micron. The filters which solved this lint problem can be obtained but they are not generally on sale.

It is true that almost all patients, I will say 70 or 75 per cent, which you remove to a hospital environment, will clear up

THE CONTRIBUTION OF AIR ANALYSIS TO THE STUDY OF ALLERGY*

COMPARATIVE RAGWEED RECORDS FOR NINE LARGE CITIES

By O C DURHAM INDIANAPOLIS IND

CLINICAL records have long given evidence of great fluctuations in severity of symptoms experienced by hay fever sufferers. These daily and seasonal variations have been difficult to understand and quite impossible to anticipate though the factors that contribute to the situation have long been recognized in a general way. It is known that a particularly favorable growing season produces more luxuriant weeds and consequently abundant pollen, and that extreme weather conditions such as a prolonged period of rain or very windy weather, have a direct effect on pollen distribution and hence upon the comfort of the hay fever victims. The comparison of weather records with clinical records is of some help. But since the whole question seems to revolve around the point of the relative amount of pollen encountered day by day the need of quantitative data has long been apparent. If one can feel certain of the numerical strength of his enemy he has already laid the groundwork of his defense. Certainly in a task which is *all* defense, the advantage of accurate statistics can hardly be overestimated.

The practical value of atmospheric pollen records depends on the simplicity of the technique and the scope of approximate accuracy of the results. The technique described below is certainly simple enough when one does not try to identify more than a few kinds of pollen. Since ragweed pollen is by far the most abundant in most parts of the country it is possible for any careful technician to obtain reliable ragweed data the first season. The only real interference with the accuracy of the results is the large number of air borne soot particles encountered in some places. When this difficulty is encountered a shelter should be arranged that will not interfere with the pollen counts. If this cannot be done, it will be necessary to make the exposures at some more favorable point.

There has been some question as to the practical value of results of counts made from a single station as it was thought that conditions even in

different parts of a city due to wind currents, wind direction, and the irregular distribution of vegetation would necessitate numerous counting stations to arrive at any accurate conclusion. In this we have been surprised greatly. Scheppegeirell¹ found that pollen is encountered in fairly even concentration up to a height of a mile. Rowe² exposed plates at different levels of the eighteen story City Hall in Oakland and obtained practically the same counts at all levels. Koessler³ and I found in Chicago that we got almost the same count on a given day from a tall building down town as from the ground in the suburbs. In fact the daily results from five stations were quite similar. This was verified in a very striking way the past season by counts made independently by Duke⁴ and myself in Kansas City, at points ten miles apart, the station of the former being 200 feet above the street in the heart of the business section and that of the latter in the suburbs. It is therefore feasible to conduct quantitative pollen studies, at least for the more abundant pollens, from one station in each center of population. The record obtained will represent the approximate condition over an area with a ten mile radius. Of course this will not apply to pollens whose source is localized or whose total output is small.

The need of comparative data from various parts of the country is becoming increasingly evident. During the last two years considerable effort has been made to interest allergy men in all parts of the country in this work, and as a result we are now able to add some interesting data to that already published and present a comparative ragweed study of nine cities in widely separated parts of the country. In several of these places only one survey has been made. For these great accuracy is not claimed, but their local and comparative value is still considerable. Particular mention is made of the courtesy of each local investigator in permitting us to use his data. Credit is given below.

TECHNIC

An ordinary microscope slide is coated with white vaseline and exposed face up in a chosen location for twenty-four hours. Under convenient low power the total number of pollen grains of the variety under investigation is then counted on a unit area and the average calculated for one square centimeter. Scheppegeirell's⁵ formulas are then used to calculate the average number of pollens per cubic yard of air for the twenty-four-hour period. It is not always practical to use the same power of magnification or the same unit area for counting, but tables and formulas are available⁵ which permit of easy disposal of the calculations. For this survey "ragweed" includes pollen of all members of the ragweed family—*Ambrosia trifida*, *Ambrosia aptera* and some *Iva ciliata*, *Iva xanthifolia*, and *Xanthium*, and in California possibly some *Franseria*.

Materials—Microscope with mechanical stage, plain glass slides, white vaseline.

Exposure—Slides are coated with a thin even film of vaseline and exposed for twenty-four hours, face up in selected location with proper protection from direct wash of rain and fall of soot.

Counting—For ragweed a low power field 14 mm wide is used, and beginning at the edge of the slide, a count is made of all pollens encountered in crossing the slide (25.4 mm). The slide is then shifted sidewise and another strip across it is counted. The total count for five such trips is the approximate number of ragweed pollens per cubic yard of air for the day under investigation. The width of the field can be calculated by a micrometer eye piece and ruled slide or can be approximately calculated by means of the scale on the mechanical stage. The width of the field can be varied by lengthening or shortening the length of the microscope barrel. I use 120 mm barrel length.

FLUCTUATION

Factors influencing the daily fluctuations in pollen content are briefly (1) humidity, (2) per cent of sunshine, and (3) wind velocity. Pollen is ripened in mature anthers early each morning. The process is interfered with if there is rain or excessive humidity. Sunshine is necessary at the ripening time, not only to complete the actual maturing of the pollen but to reduce the moisture on the blossoms and in the air. Wind is all important as the distributing agent, and the greater the wind velocity, other factors being equal, the greater the concentration of pollen will be. It will be seen from the graphs that no factor or combination of factors has been sufficient to prevent distribution for a twenty four hour period after the season really begins.

COMPARISONS

The simplest items of information furnished by an air survey are the date of appearance of pollen in the air, the date of maximum distribution, and the length of the season. Ragweed pollen begins to appear on the plates in most localities early in August, but the average date of effective concentration seems to be about August 15. The length of the season is a matter of latitude,

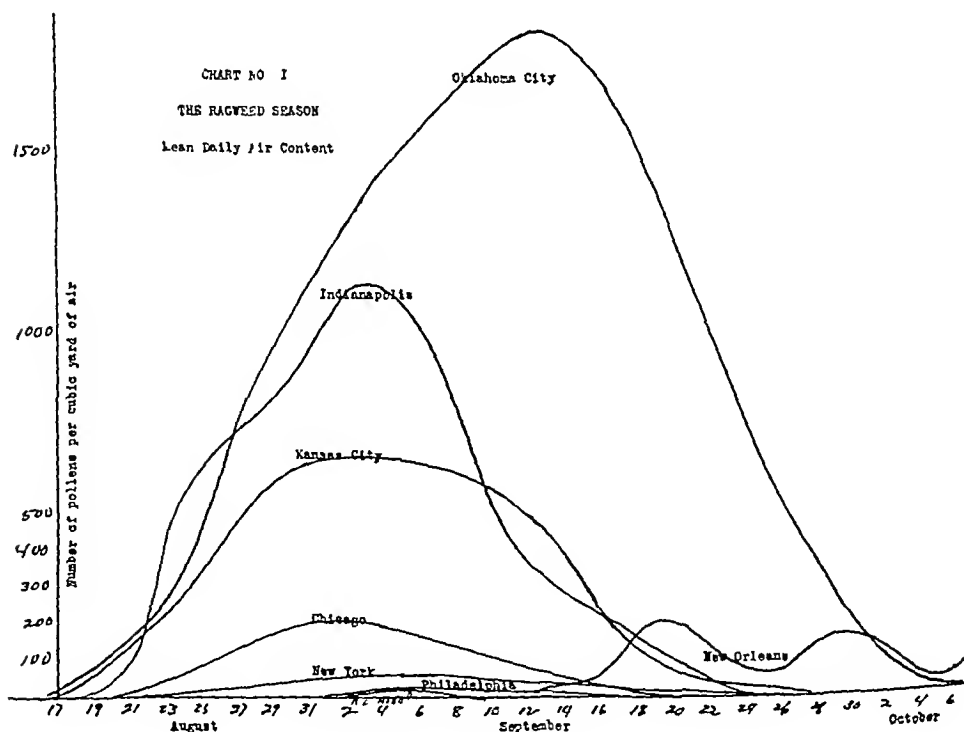
TABLE I

CITY	MAXIMUM CONCENTRATION	DAILY AVERAGE CONCENTRATION
Oklahoma City	3600 pollens per cu yd	814 pollens per cu yd
Indianapolis	2100 " " " "	470 " " " "
Kansas City	2237 " " " "	400 " " " "
Chicago	250 " " " "	100 " " " "
New Orleans	410 " " " "	75 " " " "
New York	100 " " " "	26 " " " "
Philadelphia	38 " " " "	10 " " " "
Richmond	12 " " " "	6 " " " "
Oakland	6 " " " "	1 " " " "
Average Midwestern City (Oklahoma City Indian apolis Kansas City)	2646 " " " "	561 " " " "
Average Eastern City (New York Philadelphia Rich mond)	50 " " " "	14 " " " "

and where the season is long the date of maximum pollen counts is relatively later than where the season is short. In the northern cities the peak is found to come soon after September 1, in New Orleans late in September, and in California about October 10.

The greatest surprise encountered in studying the tabulated results of these nine surveys is the astonishing difference in maximum, average, and mean daily concentration in the different cities. These differences are so great that it is impossible to plot all the actual findings on the same scale. Maximum and average comparisons will be found in Table I. Comparison of average daily concentration is also shown in Chart II. Mean daily record is shown by smoothed curves in Chart I, and even thus it is impossible properly to represent New York, Philadelphia, Richmond, and California counts. In the latter part of this article all local records are drawn on suitable scales. We find the maximum concentration in Oklahoma City at the height of the season to be 300 times that of Richmond's highest count. For purposes of comparison, the figures for daily averages probably best represent the true conditions. The daily average concentration in Oklahoma is 136 times that of Richmond and the average daily concentration in the Midwest 40 times that of the East. It may then be said that with our present information the ragweed pollen incidence is 40 to 300 times as great in the midwestern cities as it is in the eastern cities.

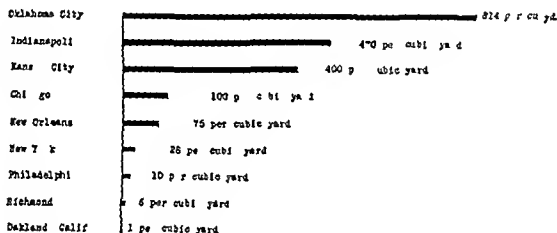
Balyeat⁶ has called attention to the fact that no differences in the weather factors entering into the production and distribution of ragweed pollen in these various places could possibly account for such extreme differences in air content, so we are forced to conclude that the amount of vegetation is the chief factor influencing the intensity of the ragweed season in various places. If these eastern records are typical, it seems fortunate that these centers of population are not exposed to the concentration of ragweed pollen that is encountered in the midwest. Since the three eastern records here included



were all made during the same season by five careful technicians working independently but following identical technique we are inclined to believe that they are representative of the true conditions. Whether there is actually a lower percentage of hay fever sufferers in the East than in the Midwest is not known. We do know that the fall hay fever symptoms are mild in Oakland, California, and with the report of Dr. Rowe, we seem to have reached the minimum toxic ragweed concentration.

In reading the charts accompanying the following surveys, it is very important to notice that it was necessary to use three different scales none of which is the same as on Chart I.

CHART NO. II
COMPARISON OF DAILY AVERAGE RAGWEED
POLLEN CONCENTRATION



OKLAHOMA CITY, 1926-1927 (CHART III)

Records by Ray M. Balyeat, M.D.

The Oklahoma City records were made from plates exposed in the suburbs of the city. No doubt ideal weather conditions had considerable to do with the extremely high counts in 1926, but the really important factor was the unusually favorable spring and summer in that part of Oklahoma. The state enjoyed record cotton and corn crops—and record ragweed crop. Vegetation was not burned up in the summer but frequent rains allowed it to reach its maximum development. Added to the favorable growing season all weather factors continued to be combined through the ripening season causing unusually heavy distribution of the pollen. Scale is one half that of Chart I.

INDIANAPOLIS, 1926 (CHART IV)

Record by Thurman B. Rice, M.D.

Plates were placed on the roof of a downtown building (Merchants Bank Building) 300 feet above the ground. Some difficulty was encountered with smoke and soot, but it is interesting to study data made in the heart of a city of 350,000 people. The fluctuations of the curve are more regular than on any other chart yet encountered. Same scale as Oklahoma City.

KANSAS CITY, 1926-1927 (CHART V)

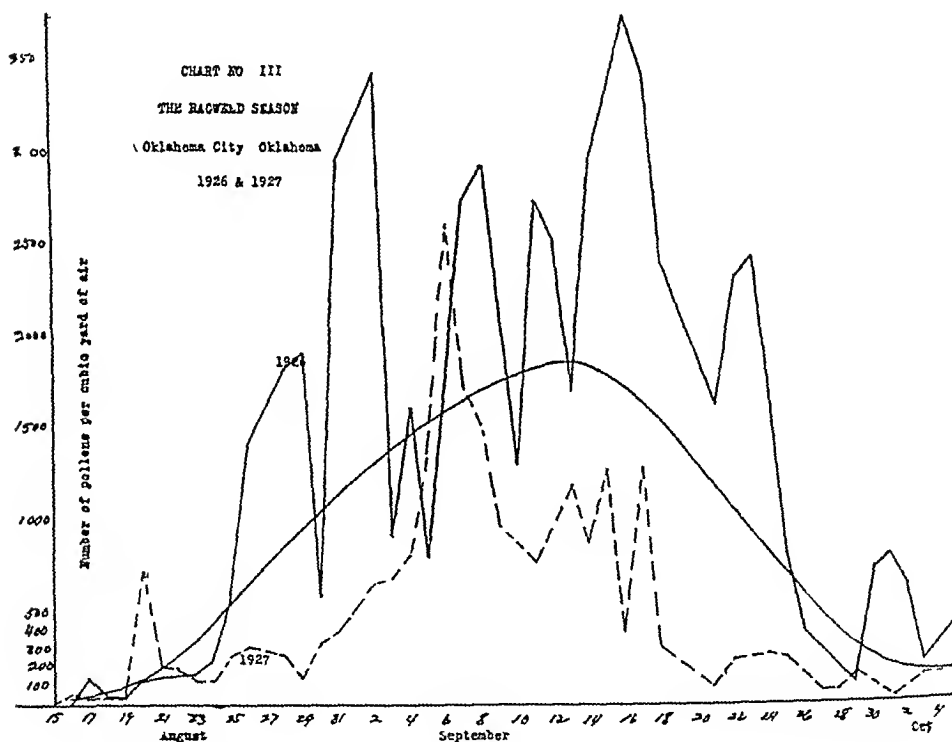
Records by O C Durham

The plates were exposed in the extreme southeast part of the city The appearance of the 1926 curve is affected most by the two days of greatest pollen incidence coming not in the middle of the season, but two weeks apart It is evident that our conclusions are much more accurate in these cities where we have data for two seasons Same scale as above

CHICAGO, 1925 (CHART VI)

Record by K K Koessler, M D ³

In this survey the data came from an average of four stations The stations were established at widely separated points, the situations representing the utmost variation in actual conditions under which pollen is encountered



in the city We felt that weather conditions were especially unfavorable, but without subsequent data we are unable to say how nearly the record represents the average condition of Chicago As stated before, we did find that the daily concentration was practically the same at all stations Scale same as above

NEW ORLEANS, 1916-1918-1922 (CHART VII)

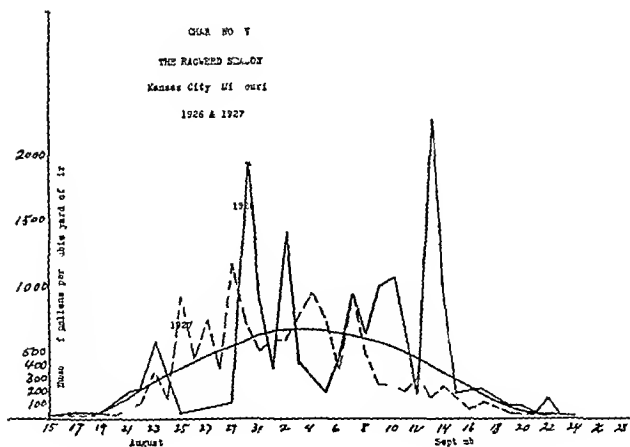
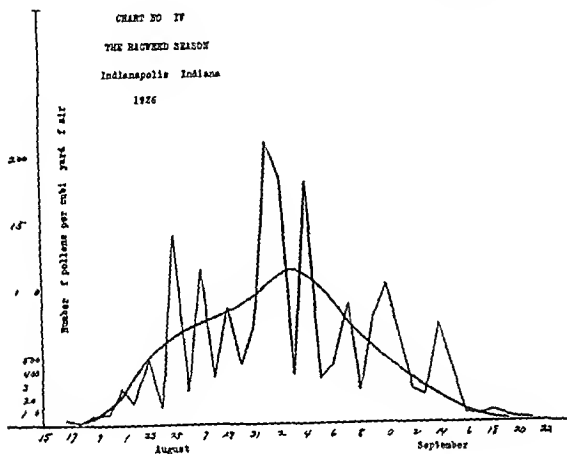
Record by Wm Scheppegegrell, M D ^{5, 8}

The average pollen concentration of New Orleans is comparable to that of Chicago The length of the season and the late climax compared with northern cities is interesting Note change of scale, 4 times that of Chart I

NEW YORK CITY, 1927 (CHART VIII)

Record by M M Peshkin, MD²⁰

Plates were exposed at 562 West End Ave. It would seem that the pollen situation on Manhattan Island is not very serious and if it were not for the succeeding surveys we would be inclined to think that there had been some mistake in the calculations. However, these have been checked and rechecked



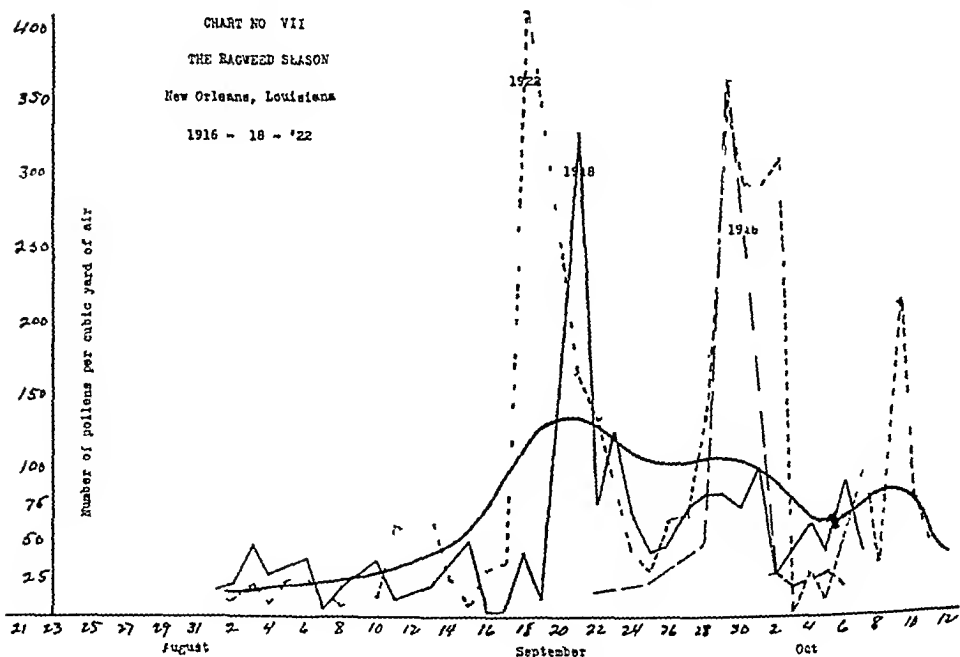
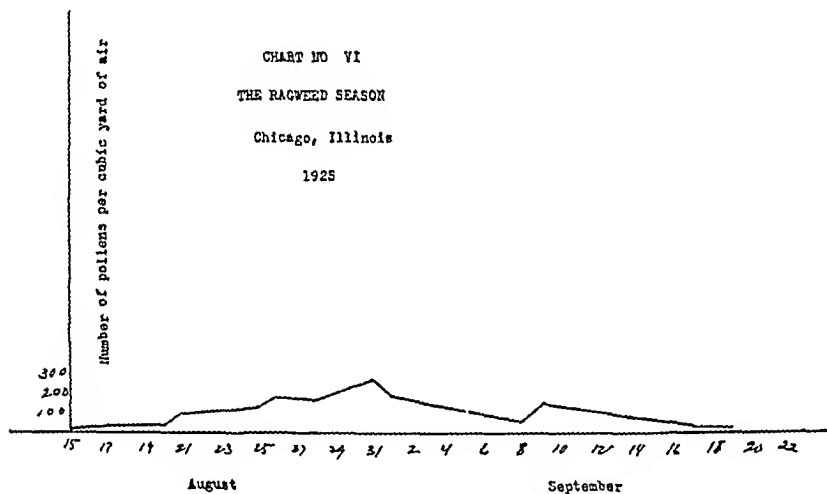
and after granting a most liberal factor for error the results are remarkably small compared with our midwestern statistics Scale 20 times that of Chart I

PHILADELPHIA, 1927 (CHART VIII)

(Two Independent Records)

By J Alexander Clarke, M D,¹¹ and H B Wilmer, M D¹²

Dr Clarke's plates were exposed at Swarthmore, a suburban town ten miles west of Philadelphia Dr Clarke feels that the season was milder than usual Dr Wilmer conducted two surveys, one in Philadelphia and one in the suburb of Germantown Data for the former was obtained at the Pres

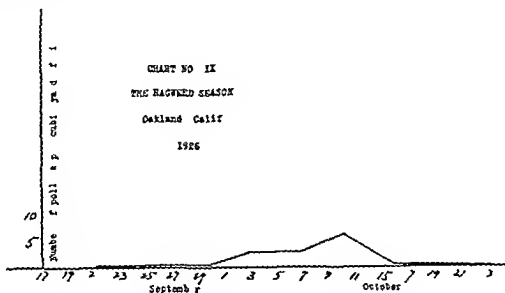
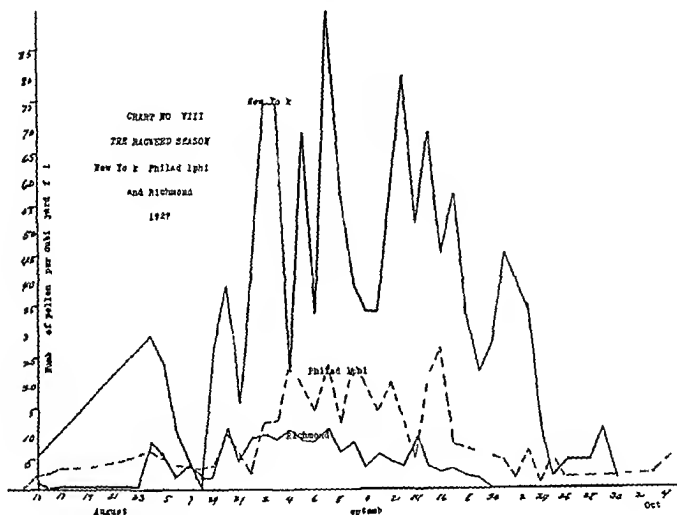


byteriau Hospital by Dr Herbert M Cobe, and for the latter by Dr C A Whitcomb The graph on Chart VIII is the average of the Swathmore and Presbyterian Hospital counts, which were quite similar and both higher than that of Germantown Thus of the three eastern cities studied in 1927, we have for Philadelphia the most accurate record

RICHMOND VA, 1927 (CHART VIII)

Record by Warren T Vaughan, M D ¹³

For this record plates were exposed on the seventh floor of an office building in the center of the city and in the edge of the city As similar daily



counts resulted, the suburban plates were discontinued. While the counts are very low, the curve is typical and consistent with that of the other eastern cities.

OAKLAND, CALIFORNIA, 1926 (CHART IX)

Record by Albert H. Rowe, M.D.²

Dr. Rowe has made the most thorough atmospheric survey yet reported. It is one of the only two surveys that cover the entire pollen season, which in this case is the whole year. The ragweed figures are incidental in the report, as there is so little ragweed in the vicinity of Oakland, but it is here included to make this article complete for all published ragweed data and because it furnishes a real minimum for comparisons. Scale of graph the same as for the eastern cities on Chart VIII.

CONCLUSIONS

- 1 Daily atmospheric pollen records afford practical quantitative data useful locally in planning hay-fever treatment.
- 2 Average ragweed pollen incidence is markedly higher in the Midwest than in the East.
- 3 There is practically no ragweed pollen in the air in California.

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- ¹²Unpublished data.
- ¹³Unpublished data.

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LABORATORY METHODS

THE NONSPECIFIC DIAGNOSIS OF ALLERGIC DISEASE FURTHER OBSERVATIONS*

BY SAMUEL M. FEINBERG, M.D. CHICAGO

THE high degree of specificity prevailing in the allergic individual has been perhaps the earliest and most constant observation made with respect to human hypersensitiveness. It has been repeatedly noted and accepted as a basic fact that when one is hypersensitive to a particular atopen an allergic reaction cannot be produced by exposure to another atopen. Similarly, the cutaneous reactivity will show a corresponding specificity. There may be considerable argument as to the theoretic explanation for this specific response, whether it is a specific ferment action or whether it is another type of antigen antibody reaction, or whether it is explainable by some other form of biologic imbalance. But there is hardly anyone who is not firmly convinced of the strictly specific nature of this phenomenon.

The conception that all allergic individuals might show a reactivity to one and the same protein would be contrary perhaps to most of the prevailing theories in regard to the mechanism of allergic reaction. However it has been found that there is at least one substance which is capable of giving a cutaneous reaction in allergic individuals to which practically all allergies show a definite skin reaction and nonallergies do not. Whether these are instances of general hypersensitiveness to this substance is rather doubtful; it may mean perhaps only a phenomenon of the dermal tissues.

In a previous communication¹ we have called attention to the fact that when 0.02 cc. of an extract of human dander is injected intradermally in an allergic asthmatic a typical urticarial reaction results whereas in nonallergies this test is negative. At that time we gave a tabulated report of 20 asthmatics who were sensitive to proteins as shown by the cutaneous tests; the human dander test was positive in all. In a small group of asthmatics who showed no positive skin tests the dander test was negative.

Van Leeuwen,² in 1922, originally described this reaction and stated further that it is applicable to all forms of allergy. Later³ he found that some of these extracts also give positive reactions in normal individuals. Such preparations must be discarded or purified by filtration through collodion membrane. In 1926⁴ he estimated that this test is positive in 90 to 95 per cent of all allergies and never in normals. As an explanation of this phenomenon he offers the suggestion that the substance present in dander causing this reaction may be the same substance present in house dust, and that it may come

Read before the American Association for the Study of Allergy, Minneapolis, Minn., June 11, 1928.

from the latter getting into the scalp Van Leeuwen believes that the reactive material is composed of animal and plant parasites, particularly *Aspergillus fumigatus*⁵ He further suggests that reactions to animal hair or dander may be due to the same causative factor

Keller⁶ reports that the van Leeuwen test was positive in all of a series of 21 cases of the type of eczema which he terms "spatexudatives Ekzematoid" He also mentions the fact that out of 2 cases of hay fever, one showed a positive reaction, and in 2 cases of urticaria the test was negative

METHOD

It is my purpose in this communication to report the results of this reaction as carried out on a greater number and variety of cases The material was prepared in the following manner To 1 gram of fresh human dander was added 100 c.c. of physiologic salt solution and allowed to stand twenty-four hours The filtered solution was then sterilized by Berkefeld filtration, tested for sterility, and sufficient phenol for a final concentration of 0.5 per cent added Before any batch of extract was accepted for use it was necessary to obtain a positive reaction in several previously proved allergic individuals and to show an absence of reaction in a number of nonallergies I have found it more suitable to use 0.02 c.c. of the extract instead of 0.05 c.c. as suggested by van Leeuwen This amount is injected intradermally on the forearm, using a control injection of phenolized salt solution on the opposite arm A positive reaction consists of a definite urticarial wheal with surrounding erythema and usually associated with itching This occurs usually in five to ten minutes

RESULTS IN BRONCHIAL ASTHMA

The cases reported here include those reported in our previous communication All of the cases reported in this paper were completely tested with the usual protein tests by the cutaneous method The results are summarized in Table I

TABLE I

	NO OF CASES	VAN LEEUWEN TEST	
		POSITIVE	NEGATIVE
Asthma	134		
Total sensitive cases	94	94	0
Sensitive to proteins other than house dust	72	72	0
Sensitive to house dust only	19	19	0
Sensitive to foods only	3	3	0
Slightly positive to house dust	2	0	2
Negative to all proteins, including house dust	40	0	40
Hay Fever, total	38		
Uncomplicated	21	17	2 (2 sl't pos)
Complicated by other allergic disease	17	17	0
Hyperesthetic Rhinitis, total	11		
Sensitive to proteins	6	6	0
Negative to proteins	5	5	0

In a series of 134 cases of asthma in which both the complete cutaneous tests with all proteins as well as the van Leeuwen test were made the following results were obtained. In 94 cases of this series, or 70 per cent, positive tests were obtained to various proteins, of which 19, or 14.1 per cent, showed house dust as the only causative factor, and 3 showed foods as the only reacting agents. The van Leeuwen reaction was positive in this entire group of protein sensitive cases. It is interesting to note in this connection that even those cases which did not show specific reactions to the individual proteins but gave a strong reaction to house dust only showed a positive van Leeuwen test in every instance.

In this same group of 134 cases there were 40 patients in whom the van Leeuwen test was negative. This group consisted of typical bronchial asthmatics, asthmatic bronchitis, and two or three other varieties of dyspnea. In all of these complete protein skin tests were made and found to be negative. The house dust extract was found to be negative also with the exception of two weakly positive reactions. In conjunction with these experiments from time to time control tests were carried out on normal individuals or those suffering from unrelated illness. These showed negative results.

HAY FEVER

It is regrettable that I had paid little attention to this reaction in hay fever cases in past seasons. Part of this neglect is attributable to the fact that I had considered it unimportant to investigate this phase of the van Leeuwen test, as hay fever patients are known to react to proteins almost invariably and, therefore, the practical value of such a test was not apparent. However it now appears that the van Leeuwen reaction may possibly have another significance, and for this season, therefore I have thought it worth while to try this reaction on the hay fever patients.

To date I have investigated the reaction of human dander extract in 38 cases of hay fever. Of these cases, 21 were uncomplicated by any other form of allergic disease, positive van Leeuwen tests were obtained in 17 weakly positive in 2 and negative in 2. One of the negative reactions was in a patient who had had three years of adequate treatment and who gave a negligible reaction to pollens this season. One of those giving a weakly positive van Leeuwen test also showed weak reactions to pollens. In the other 17 cases the hay fever was complicated by other allergic disease such as bronchial asthma, hyperesthetic rhinitis or urticaria, the van Leeuwen test was positive in all of these. As a whole, the reactions in the hay fever cases were not as strongly positive as in the asthmatics.

HYPERESTHETIC RHINITIS

In a series of 11 cases of hyperesthetic rhinitis, uncomplicated by any other allergic disease, in whom complete protein tests were made including the van Leeuwen test, the following results were obtained. In 6 positive skin tests were obtained to some proteins, two of which were sensitive to house dust only. All of these proved allergic cases gave a positive van Leeuwen reaction. In the 5 who gave no skin reaction to any protein, the van Leeuwen test was negative.

URTICARIA, ECZEMA, ETC

At this time I am unable to present any report on any series of cases of urticaria or eczema in which complete skin tests were checked up with the van Leeuwen reaction. From the occasional case in which I have had the opportunity to do this my impression is that the human dander extract reaction goes hand in hand with the specific protein reaction, i.e., if there is any cutaneous reaction with any protein there will also result a positive van Leeuwen test. However, it would require more extended observations to give a decided opinion on this phase of the subject.

It would seem logical to attempt to investigate this reaction further in such conditions which are claimed by some to be of allergic origin, such as migraine, angioneurotic edema, some forms of arthritis, and others. This is now under process of investigation.

HAIR EXTRACT

In order to determine whether this reacting substance is present in greater concentration in the dander or the hair, fluid extracts of human hair were prepared by a similar technique as that used for dander. On several patients comparative tests were made with the dander and hair extracts. Although occasionally a patient gave a strongly positive reaction to the hair extract, most reactions were very weak or entirely negative.

It is also worthy of note that in almost every instance in which the van Leeuwen test was positive and in which I have tried the intradermal house dust extract the latter was also positive. However, very many of these house dust reactions were not as strikingly positive as those produced by the dander. Nevertheless, it is possible that we have also in house dust the same or similar substance that is present in human dander and which is responsible for the skin reaction.

DISCUSSION

The results obtained in the foregoing experiments would indicate that the intradermal test with extract of human dander is a valuable means of determining the patient's reactivity to protein substances. Whether or not it can be of use in allergic individuals who do give specific cutaneous reactions has not been settled. It is unquestionably, therefore, a great diagnostic aid.

An attempt to explain this reaction on the basis of our conception of hypersensitiveness suggests several possibilities. It may be argued that these cases which react to human dander extract have become hypersensitive to human epithelium. This, of course, is not in agreement with our clinical observations. Another possible explanation is that the individual has become hypersensitive to a specific substance which attaches itself to the epithelium. Van Leeuwen thinks that this substance is the allergen of fungi, particularly *Aspergillus fumigatus*. It would be difficult to explain, however, how all allergic patients can become specifically hypersensitive to the same protein.

The contention that this reaction may be due to the presence of toxic split-protein products is open to argument because we would also expect a similar reaction in nonallergies. However, the possibility that the variation

between allergies and nonallergies may be only a quantitative difference has not been investigated and may perhaps not be contrary to our split protein theory. In substantiation of this it has been recently shown⁷ that the human skin when extracted with alcohol contains a substance that flushes and wheals the human skin when punctured into it, and that the epidermis contains this substance in greater concentration than the dermis.

SUMMARY

1 An extract prepared from human dander and injected intradermally shows a positive skin reaction in practically all individuals manifesting cutaneous reactivity to proteins.

2 In a series of 183 cases of bronchial asthma hay fever and hyperesthetic rhinitis, in which complete protein skin tests were made, with positive results in 138 and negative in 45 the human dander test failed to show agreement in only 4 instances.

3 It is suggested that there may be a valuable diagnostic feature to this reaction, not only in the conditions described but perhaps also in other manifestations in which the etiology of allergy is still in debate.

4 It is possible that there may be found other materials containing substances capable of giving a similar reaction.

5 The occurrence of such a reaction in all allergic individuals without regard to the specific protein to which the individuals are hypersensitive would tend perhaps to invoke a modification of some of our conceptions regarding specificity in hypersensitiveness.

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30 NORTH MICHIGAN BOULEVARD

DISCUSSION

Dr M Murray Peshkin New York City—Two years ago I tested fifty children with allergic asthma to an extract of human dander and another fifty children with *Aspergillus fumigatus*. The intradermal and scratch tests were performed in every case. The preparations were kindly furnished by Dr Van Leeuwen of Leiden, Holland. I can state now that in only a few instances were positive reactions obtained with either of these preparations.

The following experience will be of interest to note. One and one-half years ago a female patient, a manicurist by occupation, came under my observation for bronchial asthma. Her attacks of asthma were always aggravated and frequently induced on the days when much hair clipping was performed in the barber shop where she was employed. She showed strongly positive scratch reaction to both the fluid and powdered extracts of human hair and dander. Positive reactions were also obtained to a number of other protein substances. A subcutaneous injection of 0.1 cc of a 1:10,000 dilution of human

dander caused a violent local reaction persisting for several days. I felt justified in assuming that I was now dealing with a definitely potent extract of human dander. With this extract over 100 patients with asthma were tested intradermally and positive reactions were obtained in only a few instances.

In a patient with asthma exhibiting multiple positive protein skin reactions the problem for the investigator is to determine which protein or proteins are the existing etiologic factors. If this is not accomplished then from a practical standpoint the knowledge that the patient is allergically sensitive is of no value. The knowledge that such a patient also reacted to human dander would likewise be of no practical value. However, if we had it our disposal a single remedy, perhaps a complex synthetic chemical which when injected into an allergic asthmatic would alter the reactivity of the host in such a manner as to render him free from symptoms in spite of exposure to a known exciting factor of asthma, then any single test that will enable one to make a positive diagnosis of allergy would be of unquestionable value. Such a test, however, must pass all suspicion of not being nonspecific in nature and must not give pseudoreactions. In the light of my experience I cannot accept, at this time, the van Leeuwen test as a specifically diagnostic test for allergy.

Dr Ray M. Balyeat, Oklahoma City, Okla.—Last fall about the first of October we began intradermal testing with human dander made up as Dr. Feinberg makes his and I must say that our experiences differs very materially from Dr. Peshkin's, and conforms largely with the experience of Dr. Feinberg. We have run a series of something over 250 in which we found that in those patients who are having migraine or urticaria that the reaction to human dander is seldom positive, while in the majority of cases who are sensitive to pollen only very mild reactions to human dander occur, but in practically all cases who are very sensitive to animal dander the human dander is very strongly positive.

In the collecting of human dander it is very interesting to note that it is not easily obtained, although we frequently think of human dander as being very prolific. However, it does not seem so when the actual collecting is taking place. I might say, in regard to our collecting, that it is obtained from the heads of our insane people, and that it can be obtained, as a rule, in large quantities from patients suffering from encephalitis lethargica.

Now in regard to its practical value, I think it is going to be of some value. For example, two or three patients came into the office and after taking the history I thought that they were entirely hypertension cases suffering from cardiac decompensation with asthmatic symptoms, simulating true asthma. On testing them first with human dander we found them strongly positive, which led us to go further with our tests, and they proved to be true asthmatic cases complicated with hypertension. It seems to me that if one finds patients strongly positive to human dander that we are justified in searching very carefully for an allergic factor as a cause.

Dr. Leon Unger, Chicago, Ill.—Dr. Feinberg and I are associated with the Northwestern Clinic, and we do very complete skin testing, running through 200 to 250 tests on each case. We have been running both the allergic, and the cases we deem nonallergic through the same routine of skin tests.

The value of the van Leeuwen test seems to lie in the elimination of the necessity of skin testing in the nonallergic group of cases. We have found without exception, as Dr. Feinberg has pointed out, that every single case that has had negative skin tests has been negative to the van Leeuwen test. This group includes cardiacs and so called asthmatic bronchitis and other patients.

We have also found, as Dr. Feinberg pointed out, that practically every single case of allergic patients with one or two exceptions has been positive to the Van Leeuwen test.

I know Dr. Feinberg has been very conscientious in this work and he has discarded several batches of material. If he gets a batch and tests it out on normals and finds that the normals give positive, he immediately discards the batch of material and runs some more. If he finds that the allergic cases give negative reaction, he discards the batch and prepares fresh ones, as he explained. Then when he gets a batch which gives negative skin

tests on normal individuals, students and nurses and so forth and gets a batch which gives positive reactions to allergic cases, those who give positive skin tests then that is the batch we use for testing for the patients

Our results at the clinic have been very gratifying and I cannot understand why Dr Peshkin has not obtained satisfactory findings. I am sure that the work will go on and at the next meeting Dr Feinberg will probably have more to report on this subject. In the meantime, the test offers us a tremendous help in eliminating the necessity of skin testing the nonallergic group of cases. If we find a heart case or a hypertension case, we immediately do a van Leeuwen test. If the test is negative as it practically always is we know these patients are nonallergic, and we don't have to run through 200 or 250 skin tests which our experience has shown always prove negative in a particular group of cases. Up to the present time we have been testing these cases thoroughly but we are contemplating dropping the skin tests in those patients who give negative van Leeuwen tests.

Dr Samuel M Feinberg Chicago Ill—I have tried to convey the impression that this particular reaction is positive in individuals that give reactions to other proteins. I did not mean to convey the impression that if it is negative the asthma is nonallergic, as a patient may still be allergic and not react to proteins. Apparently, these do not react to the van Leeuwen tests. In other words, the same influence affects the skin, which apparently influences it to reactivity to the van Leeuwen test thus my series of cases really is a comparison of the specific cutaneous tests to this intradermal human dander.

I do not know why Dr Peshkin has not been able to corroborate this work. There are possible explanations for his failure. That particular specimen which he got from van Leeuwen may have been old. I have found very frequently when I have had an extract for two months it fails to work or fails to react very much. I have made it my practice to make a new batch at least every month. That is one thing.

Another thing is that he is dealing, as I understand it almost exclusively with children and we know children do not give the same or equal cutaneous response that adults do ordinarily. Positive cutaneous responses in children are frequently of a degree that we would call negative in adults. Still we can say they are of significance in children. It may be the interpretation in children is something different from that of adults as far as the positivity of a test is concerned.

As to the practical value of the test, Dr Peshkin again fails to see its importance. I think that that point has been covered by Dr Unger in his discussion that when I get a negative test in my estimation at least I am quite certain that I am going to fail to get any specific cutaneous reactions. I have not failed to make the individual protein tests in all of these cases nevertheless for the sake of control and experiment. Every time I get a new patient whether it is hyperesthetic rhinitis or asthma, if I have the proper extract I make an intracutaneous test, and if it proves negative I very often tell the doctor or the patient I do not think it advisable to go ahead with the tests. If the doctor wishes it, that is all right. If the patient insists that is another matter.

Invariably I have found I could foretell negative skin reactions. I have not had the opportunity to test out any possible allergies who do not give cutaneous reactions. Whether this test would react on them or not I am not sure.

I am glad to hear that Dr Bulgeat has in a great measure corroborated my findings.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M D, ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

BONE CYST The Etiology of Solitary Bone Cyst, Phemister, D B, and Gordon, J E
Jour Am Med Assn, October 30, 1926, lxxvii, 1429

The clinical picture of this case was definitely that of an infectious disease. However, the gross and microscopic pictures of the lesion in the humerus were practically typical of a bone cyst. The very large number of giant cells producing extensive areas in the lining membrane that were almost identical with benign giant cell tumor gave the tissue an aspect that was markedly different from that seen in ordinary pyogenic osteomyelitis. At the same time, there were leucocytes in sufficient numbers to characterize it as inflammatory. The tibial lesions also contained large numbers of giant cells, but there the leucocytic infiltration and exudation were much more extensive.

The microscopic picture simulating giant cell tumor suggests a possible inflammatory nature for that disease and the possibility that bone cyst in childhood is from the same cause as benign giant cell tumor, which occurs almost entirely in adults. Cases are seen in late adolescence or early adult life, in which it is difficult to say whether the lesion should be classified as bone cyst with a thick fleshy lining or benign giant cell tumor which has undergone cystic degeneration. Benign giant cell tumor has often been regarded as a chronic inflammation, but as yet there is no bacteriologic evidence to substantiate the view.

The evidence in these two cases is too incomplete to be conclusive, but it is strongly suggestive that *Streptococcus viridans*, isolated in each instance from the bone cyst, was the cause of the disease. Animal experiments were insufficient in one case and unsuccessful in another. The fact that the microorganism is the same as that found by Gilmer and Moody, Rosenow, Haden and others in chronic periapical dental infections, which usually develop silently, producing extensive bone absorption and frequently large pockets without surrounding new bone formation, lends support to this view.

TUBERCULOSIS A Comparison of the Pirquet, Mantoux, Ring, and Tubercumet Tests in Tymanhurst Children, Stewart, C A, and Collins, A E. Am Jour Dis Child, September, 1926, xxxii, 367

In healthy children having negative tuberculin tests, the ring and tubercumet tests are uniformly negative.

In children not suffering from acute infections, but having positive Pirquet and Mantoux tests, together with such clinical findings as enlargement and calcification of the bronchial lymph nodes, slight occasional elevation in temperature, undernutrition and a history of exposure to tuberculosis but with no evidence of clinically active tuberculosis, the ring and tubercumet tests are negative. Under such circumstances, negative ring and tubercumet tests apparently are confirmatory evidence that the tuberculous focus is inactive and thus of definite clinical value.

In definitely active tuberculosis the ring and tubercumet tests usually are positive.

Positive ring and tubercumet tests are not specific tests for active tuberculosis, for they may become temporarily positive as the result of acute nontuberculous infections.

To be of significance, the positive ring and tubercumet tests should be persistently positive. Under such circumstances when certain chronic nontuberculous infections, such as pyocystitis, osteomyelitis, and others are excluded, persistently positive ring and tubercumet tests should cause one to suspect strongly the presence of active tuberculosis.

A diagnosis of active tuberculosis on the basis of positive ring and tubercumet tests alone should not be made.

In making a diagnosis of active tuberculosis, the results of the ring and tubercumet tests should be used with and not to the exclusion of other established methods of examination

Tests, such as the ring and tubercumet reactions, which are known to be positive in cases of active tuberculosis and negative in healed tuberculosis, undoubtedly will prove of value when their limitations are fully understood

The Intracutaneous Salt-Solution Wheal Test, Stern W G and Cohen M B Jour Am Med Assn, October 23, 1926, lxxvii, 1355

In the absence of edema, the intracutaneous salt solution test is a simple rapid, and accurate method of determining circulatory deficiencies in the extremities

Sixty minutes or more is the normal disappearance time of the salt solution.

In all instances in which clinical circulatory deficiency exists the disappearance time is diminished, in the area just above the seat of gangrene (existing or threatened), it is frequently as low as five minutes

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

Works on Allergy

UNFORTUNATELY the major portion of the available contributions on allergy both experimental and clinical are widely scattered through a large number of current periodicals. The student of this subject is therefore faced at the outset with considerable difficulty in the building up of his reference library. At the same time there are many excellent monographic contributions which as a whole make a very good three foot reference shelf.

Many of the earlier works dealing especially with fundamental principles are long since out of print and therefore need not be mentioned. Two of these however are certainly deserving of a few remarks. Blackley's original book written in 1873 is an inspiration to all who have an opportunity to read it. In his *Experimental Researches on the Causes and Nature of Catarrhus Acutus Hay Fever or Hay Asthma* we find that he has anticipated most of the methods which we employ today. In this book we find a method for pollen plating and counting very much as we use it today and a pollen prevalence curve which is directly comparable to the many which have appeared within the last few years.

The other of the out of print works which should receive especial mention is Vaughan's *Protein Split Products in Relation to Immunity and Disease* for in the reviewer's opinion an acquaintance with the contents of this book and the viewpoint of the author gives the reader a clearer understanding of the fundamental processes of anaphylaxis and allergy than does any other of the older works. It is true that newer theories have since been proposed, the terminology of which conforms more to the more recent knowledge gained particularly from a study of colloidal chemistry but so far no new basic theory has received generalized recognition or supplanted the older theories summarized in this work.

Among the works on clinical allergy Duke's *Asthma, Hay Fever, Urticaria and Allied Manifestations of Allergy* and Scheppegegrell's *Hay Fever and Asthma* should still find a prominent place on the book shelf of every allergist. Duke finds allergy a factor in a remarkable diversity of clinical conditions. Since the appearance of his book nearly all of these observations have been confirmed by other workers. This is the only work in which any great amount of attention has been given to Physical Allergy.

Scheppegegrell deals almost entirely with pollen allergy. The reviewer feels that his book should be widely read particularly because the botany of allergy is more comprehensively given than in any other monograph.

On the other hand since this work was published the list of hay fever producing plants has received many additions and some revision. The second edition of Balyeat's *Hay Fever and Asthma* contains a large section devoted to botany and is the only work in which the list of hay fever producing plants is entirely up to-date.

Balyeat's book was written primarily as a manual for the use of the patient and can be highly recommended as such but at the same time it is a book that may be read with profit by the physician and indeed by the allergist.

Another 1928 addition to the allergy shelf is Thomas' *Asthma*. It contains minutiae which are of service to the beginner but its chief value to the student of allergy lies in the

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

author's detail of his methods for bacteriologic study and treatment. For Thomas is one of the group who are confident that specific bacterial sensitization plays a part in asthma.

Two volumes which are always desirable as reference works and between the covers of which a wealth of information is contained are Kolmer's *Infection, Immunity and Biologic Therapy* which has a large section devoted to clinical allergy, and Zinsser's *Infection and Resistance*. Another much smaller volume which should find a place and which has likewise already been reviewed in these columns is Wells' *Chemical Aspects of Immunity*.

We can say quite truthfully that the outstanding recent advances in the study and treatment of clinical allergy have been made in this country. We do say also with some degree of truth that interest in the subject in Great Britain and on the Continent has lagged far behind American interest. But there are certain important exceptions. In France interest in anaphylaxis has never lagged. True until recently at least skin testing has not been prosecuted as vigorously as on this side of the Atlantic but there are few phases of anaphylaxis and allergy in which the contributions of French authors will not receive considerable mention.

Charles Richet who in 1902 coined the word anaphylaxis published in 1925 a fairly complete review of work that has been done in the intervening twenty years and brings his conceptions of anaphylaxis up to date. Little mention is made of clinical allergic manifestations, his interest being primarily in experimental anaphylaxis but this in no way detracts from its interest to the allergist.

Among other relatively recent French contributions well worthy of possession are Lumière's *The Problem of Anaphylaxis* in which he presents his theory of colloidal precipitation, Arthus' *From Anaphylaxis to Immunity*, Danysz' *Theory of Immunity, Anaphylaxis and Ananaphylaxis*, Minet and Leclercq's *The Practical Applications of Anaphylaxis* in which the authors discuss such conditions as serum sickness, drug idiosyncrasy, echinococcus sensitization, anaphylaxis in infectious diseases, diagnosis of cancer of echinococcus, infection of typhoid fever and tuberculosis and anaphylaxis in general. Lacroche, Richet and Saint Girons' *Alimentary Anaphylaxis* primarily a clinical study, and Calup and Segard's *Pathogenesis and Treatment of Asthma* which is entirely clinical.

Nor are all of the continental contributions French in origin. We should not fail to mention VanLeeuwen's *Allergic Diseases*. Incidentally VanLeeuwen has written a supplementary article in *International Clinics* for June 1927 which explains perhaps a little more clearly than does the book his conception of so called masmatic asthma.

Frank Coke has stressed the importance of studying asthma as an allergic manifestation as much as any other man in England. His experiences have been collected into his book on *Asthma*. This is a comprehensive exposition of the subject. The author's classification as to types is original and interesting. He classes asthma into the protein sensitization types: the thin type, the week end type, the day or night type, the type combining asthma with colds, the local type, the periodic, the menstrual, the occupational, the drug sensitive, the bronchial type, the type associated with rapid heart and the colloidolysis type.

The author adds a new term "adzyme" which to the mind of the reviewer does not clarify our understanding of anaphylaxis but which as the author suggests is for use only until a better understanding of the phenomenon has been reached. We feel that it is an unnecessary term. Another section that should interest the American reader is his discussion of vaccine therapy, particularly the method of ecogenous autogenous vaccine therapy that has been developed by Danysz.

The allergist who sees now large volume of eczema or dermatitis is inclined to feel that the dermatologist pays too little attention to the possibilities of protein sensitization. R. Cranston Low, a Scotchman, has written an entire volume on the relation of allergy to dermatologic lesions and one will find considerable of interest in his *Anaphylaxis and Sensitization*. His original work presenting evidence of the development of an immunity following epidermophyton infections is especially worthy of note. The work is abundantly illustrated with many color photographs.

The otolaryngologist has in many localities been likewise slow at accepting the theories of allergy. James Adam of Glasgow has written a volume on *Asthma and Its Radical Treat*

ment One may anticipate his attitude in reading in the preface that "much water and more wind, mostly anaphylactic, has flowed under the bridges since the first edition was written"

The author presents his hypothesis that asthma is primarily a toxemia due in part to putrefaction and absorption from the intestines and in part to some error in nitrogenous metabolism which is closely connected with excess of carbohydrate in the diet He claims that oxidation of the excess of the simpler carbohydrate molecule seems to interfere with proper oxidation of the more complex protein molecule He presents no convincing experimental evidence He discusses anaphylaxis in some detail and admits that there are some anaphylactic cases but on the whole he has little patience with the theory of allergy Statements such as the following might bear some argument "Idiosyncrasy to feathers does occur in which case asthma usually arises when or shortly after the patient goes to bed, it has nothing to do with the attack that so suddenly comes between two and four A M" The author marshals his evidence against allergy as the fundamental cause for asthma and this is well worth reading even though the reader may not agree with him The aim of Adam's treatment is first to detoxicate and then to prevent toxemia by insistence on a healthy mode of life While this is in play he attempts to reduce vagal irritability, and possible sources of irritation and of sepsis, especially in the nose are treated He states children can nearly always be cured by attention to teeth, tonsils, adenoids, bowels and diet, exercise and cold sponging in the morning and the avoidance generally of coddling A weekly mercurial should be given He divides adult cases into three groups, nasal cases in which elimination of the nasal trouble will go a long way to cure, digestive cases (toxic), and allergic cases This third group are according to the author in a minority

A volume of the highest value to students of bacteriology and immunology, which has just made its appearance is Jordan and Falk's *Newer Knowledge of Bacteriology and Immunology* The editors have marshalled a great galaxy of names among the individual contributors Among the articles in this volume which should be of especial interest to immunologists are those on the chemical structure of bacteria, bacterial oxidations and reductions, protein metabolism of bacteria, enzymes of bacteria, filterable viruses, the bacteriophage, elective localization of bacteria, bacteria of the intestinal and respiratory tracts, the immunologic basis for different types of infection by blood protozoa, the mechanism of phagocytosis, local and tissue immunity, control in standardization of biologic products, the origin of antibodies, nonspecific protein therapy, chemotherapy of bacterial diseases, and many other chapters

Those who are acquainted with Coca's presentation of his theories of atopy as they are found for example in Tiee's *Medicine* will be interested in his chapter in Jordan and Falk in which he briefly presents and defends his theory of atopy Manwaring presents an extremely clear and yet brief summary of Ehrlich's side-chain theory followed by criticisms of it which more recent work has raised Manwaring's own enzyme theory which he proposes as a substitute is not followed as easily as is his presentation of Ehrlich's theory

Another recent volume into which allergy enters only incidentally but which is full of information and facts for the immunologist is the work on *Filterable Viruses* edited by Rivers

Those who are interested in serologic investigations and the humoral aspects of immunity will find considerable of interest in Browning's *Immunochemical Studies* This is a compilation of work done mainly in the pathological department of the University and Western Infirmary, Glasgow, and deals with investigation in the nature of antigen, antibody and complement

Those who are delving into the possibilities and the results of clinical immunization against the bacterial diseases will find in Robertson's *Therapeutic Immunization* a presentation of the author's wide experience and conclusions Methods are described in quite minute detail

Very good information on vaccines and biologic implements of therapy is to be found in Solis Cohen and Githens' *Pharmaco-Therapeutics* The senior author presents in detail his technique and the theoretical considerations in connection with his *selective pathogen culture*

method. A great deal of information is to be found in this volume also on the pharmacodynamics of calcium and other inorganic ions. For information on biologic therapy Kolmer's monumental work should not be overlooked.

Works on Vital Processes

The student of allergy is a student of vital activity. Allergy does not occur in dead substances. The phenomena of anaphylaxis, immunity and allergy are abnormal manifestations of vital activity. For an intimate understanding of these manifestations one must have a well grounded understanding of normal activity. The following works are suggested for this purpose.

Bayliss' *Colloidal State* is an excellent introduction to an understanding of colloidal chemistry in its medical and physiologic aspects. We might term it the A B C of colloidal chemistry.

A series of lectures on *Biologic Aspects Colloidal and Physiologic Chemistry* has been published in book form by The Mayo Foundation. The first chapter contributed by Robert A. Millikan deals with Principles Underlying Colloid Chemistry. Martin Fisher contributes a chapter on Colloid Chemistry in Biology and Medicine. Robert Chambers writes on the Physical Properties of Protoplasm. Ross A. Gortner tells of Adsorption and Vital Phenomena. E. Franklin Burton covers the Physics of the Ultramicroscope and the Optical Properties of Colloid Particles. The last chapter by William T. Bovie deals with the biologic effects of life.

Dr. Robert Chambers also has an excellent paper on *The Nature of the Living Cell as Revealed by Microdissection* in *The Harvey Lectures* Series 22, 1926-27. In this same volume is a brief but excellent discussion on *Organic Chemistry* its relation to medicine by Dr. Richard Willstätter of Munich.

There is a volume which should be in the hands of every student of vital activity. This is *General Cytology* edited by Edmund V. Cowdry. Three chapters in particular deal with the subject under consideration. These are *Some General Considerations of the Chemistry of Cells* by Albert P. Matthews, *Permeability of the Cell to Diffusing Substances* by Merle H. Jacobs and *Reactivity of the Cell* by Ralph S. Lillie.

A companion work to *General Cytology* *Special Cytology* edited again by Cowdry is the best reference work available on the histology, embryology and function of differentiated cells such as are found in the various tissues of the living body. For the purpose of the present review we would recommend particularly the chapters on the *Special Cytology of the Skin*, the *Mucous Membrane of the Respiratory Tract*, the *Intestinal Epithelium*, the *Liver*, the *Reticulo-endothelial System* and the various types of leucocytes in the circulating blood.

Willstätter, mentioned above, has contributed a brief but instructive orientation monograph on *Problems and Methods in Enzyme Research*.

We must remember that in allergy and anaphylaxis we are dealing not only with the vital activity of the host but also with the biologic or vital activity of the antigens and that the biochemistry of the latter must also be studied. Buchanan and Fulmer have written a monumental work on *Physiology and Biochemistry of Bacteria* which contains a wealth of information but is very highly technical and therefore rather difficult of digestion.

Allergy—Asthma Hay Fever Urticaria and Allied Manifestations of Reaction by William W. Duke, Ph.B., M.D. Kansas City, Mo. 339 pages. Cloth. With seventy five illustrations. C. V. Mosby Co., St. Louis, Mo. 1925.

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Asthma—Its Diagnosis and Treatment, by William S Thomas, M D, Associate Attending Physician in Immunology, St Luke's Hospital, New York Twenty illustrations in black and white and six in color Cloth Pages 279 Paul B Hoeber, Inc New York 1928

A Practical Textbook of Infection, Immunity and Biologic Therapy with Special Reference to Immunologic Technique, by John A Kolmer, M D, Dr P H, D Sc (Hon) Professor of Pathology and Bacteriology in the Graduate School of Medicine, Univ of Pennsylvania, and Member of the Research Institute of Cutaneous Medicine, Philadelphia Cloth 202 original illustrations, 51 in colors Third Edition, thoroughly revised and mostly rewritten 1210 pages W B Saunders Co, Philadelphia 1924

Infection and Resistance, by Hans Zinsser, M D Third Edition Cloth 666 pages The Macmillan Company, New York, N Y 1923

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Le Probleme de L'Anaphylaxie, par Auguste Lumiere Avec 46 figures, dont 22 en couleurs d'apres autochromes Cloth Pages 237 Librairie Octave Doin, Gaston Doin, Editeur, 8, Place de l'Odeon, Paris 1924

De L'Anaphylaxie et L'Immunité, par Maurice Arthus Paper Pages 358 Masson et Cie, Editeurs, Libraires De L'Academie de Medecine 120, Boulevard Saint Germain, Paris 1921

Origine, Evolution et Traitement des Maladies Chroniques, Non Contagieuses Theorie de l'Immunité, de l'Anaphylaxie et de l'Antianaphylaxie, Par J Danysz Paper Pages 130 Librairie J B Bailliere et Fils, Paris, 1920

Les Applications pratiques de l'Anaphylaxie, par les Docteurs Jean Minet et J Leclercq Cloth Pages 94 Librairie J B Bailliere et Fils Paris 1913

L'Anaphylaxie Alimentaire, par les Docteurs Guy Laroche, Charles Richet Fils et François Saint Girons Paper Pages 95 Librairie J B Bailliere et Fils Paris 1919

Pathogenic et Traitement de L'Asthme, par J Galup et M Segard Paper Pages 262 Librairie Octave Doin Paris 1927

Allergic Diseases, by W Storm van Leeuwen, J B Lippincott Co, Philadelphia, Pa 1925

Asthma, by Frank Coke, FRCS Cloth Pages 260 Frontpiece in color and other illustrations Wm Wood and Co, N Y

Anaphylaxis and Sensitization With Special Reference to the Skin and Its Diseases By R Cranston Low, M D, FRCP With 16 colored plates and 7 half-tone illustrations Cloth Pages 384 William Wood & Co, New York

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EDITORIALS

The Nature of Bacteriophage

THE observation by Twort in 1915 and d'Herelle in 1917 of transmissible lysis in bacteria constituted a phenomenon of such novelty that nearly all bacteriologists have interested themselves deeply in its study. Many have carried their investigations to great length and have contributed abundantly to our knowledge of the process, but so far none has reached a generally accepted understanding of its mechanism. Nearly every man who has conducted extensive observations in this field has offered his own theory so that theories there are aplenty but there are none against which other observers have not raised serious objections.

D'Herelle believes that the lytic principle is a foreign filterable virus which parasitizes bacteria, destroying them by lysis. He looks upon bacteriophage as a contagious disease of bacteria. The infecting agent is an ultra-microscopic filterable corpuscle multiplying at the expense of young living bacteria some of which latter may acquire an immunity to the parasite becoming resistant to its lytic action.

Kabeshima was probably the first to disagree with d'Herelle and suggested that the lytic agent was a catalyzer produced by the digestive glands,

possibly also by leucocytes which activated a diastase normally present in the bacterial cells, the latter causing destruction of the cell. This view has few adherents.

A rather widely favored explanation was formulated by Bordet and Ciuka in 1920 who believe that the essential process is an autolysis resulting from a rupture of the equilibrium existing in the living bacterium between assimilation and metabolism. This is spoken of as a nutritive vitiation. With a breaking of the balance between anabolism and catabolism the bacteria undergo self destruction. The products of this autolytic destruction are toxic for other bacteria in the culture and when liberated incite autolysis in neighboring bacteria, thus making the lytic process transmissible. A criticism of this theory which appears to have considerable weight is that most of the evidence so far adduced tends to confirm d'Herelle's original assumption that the bacteriophage principle is particulate in nature and is not a substance in solution. Moreover there is some criticism of the manner in which Bordet and Ciuka explain the origin of the lytic principle. They obtained it through the action of leucocytic exudate of guinea pigs after intraperitoneal inoculation of *B. coli*. They suggested that the exudate releases the lytic action. But as Bronfenbrenner and others have pointed out bacteriophage appears to be as universally distributed as bacteria themselves, and it is difficult to establish that no bacteriophage was introduced into cultures along with the leucocytic extract. Bordet and Ciuka believe that bacteriophage is an autolysis arising in the bacteria from vitiated metabolic activity probably originally incited by some as yet hypothetical external influence but capable of self propagation because of the toxic stimulative action on other cells of these liberated autolytic products.

Most investigators agree with d'Herelle that bacteriophage is corpuscular and has definite mass, the estimated diameter ranging from twenty to ninety millimicrons or about the size of a protein micella. Not so many have agreed that d'Herelle has positively established his conception that bacteriophage is actually a living substance. He believes that he has demonstrated that it possesses powers of environmental adaptation of propagation and of metabolic assimilation in a heterogeneous medium. But other explanations for his conclusions have been suggested and the question whether bacteriophage is a living reproducing extra bacterial substance has not been settled.

Kuhn in 1919 made a careful microscopic study of certain bacterial forms present in cultures which appeared swollen and contained small granular bodies which he called Pettendorf bodies. After further study in 1926 he stated his belief that these bodies were a virus living in symbiosis with the bacterial cells, thus aligning himself more or less with d'Herelle. He states that he has seen the minute granules or spores liberated after the bursting of the swollen bacteria, attach themselves to new bacteria. This parasite according to Kuhn belongs in the classification of myxomycetes.

Koeb and Ziegenspeel have made somewhat similar observations and reached very much the same conclusion.

Beguet has proposed an osmotic theory of bacteriophagy. Some bacterial cells, the so called sensitive cells, absorb colloid from the surrounding medium increasing their osmotic pressure so that they swell and burst. This bursting liberates larger amounts of colloid material in the neighborhood of adjoining bacterial cells and the latter proceed to repeat the experience of the former. The osmotic equilibrium existing between the germ and the absorbed colloid or the colloids present in the medium is disturbed. Some cells are destroyed by an excess of internal pressure while others can adapt themselves to a lower pressure thereby becoming resistant.

This theory has not gained great favor. As Hadley says, such theories take undue liberties with our present knowledge of physical chemistry as applied to colloids in the bacterial cells and the power of absorption from the medium. "One might almost come to believe after reading such a presentation as that of Beguet that the living micro-organism is merely a toy, existing only to be played on by its colloidal environment, rather than a biologic entity possessing in itself an inherent directive mechanism."

Wollmann believes that the lytic element arises within the bacterial cell. In his earlier work he suggested that the reaction of bacteriolysis is due to corpuscular elements which arise from the bacterial cells themselves and are transmissible from mother cell to daughter cell or perhaps from affected cell to normal cell through the external medium. In this we find a suggestion of an hereditary tendency which may be transmitted in the offspring but not only in this way but also from one mature cell to another mature cell across an intervening environment. This is indeed a new idea.

More recently Wollmann has elaborated on his theory of hereditary factors. Wollmann believes that the hereditary factors are corpuscular in nature and that while they arise within the bacterial cell they may exist outside of the cell, thus developing a certain degree of autonomic activity and may produce in other normal cells the modifications of which they are the bearers.

Wollmann's hypothesis might be described as a cross between that of d'Herelle and that of Bordet and Ciuka. He calls into play an extracellular living corpuscular element but one which originates in the metabolic activity of the bacterial cell and incites similar metabolic derangement or rupture of equilibrium between anabolic and catabolic function in neighboring cells. The difficulty with the acceptance of this theory is the conception of the transmission of acquired hereditary factors through the external environment and the conception of a fragment or portion of the living cell taking on an autonomous existence so that it can continue to live outside of the dead cell.

Hadley suggests yet another hypothesis, one which he admits is yet unproved but has much in its favor and should be considered as a possibility when attempting to explain the results of any new experimental observations. This author aligns himself with those who believe that the evidence favors a living nature for the bacteriophagic principle. On the other hand he is inclined to accept the evidence that transmissible lysis may appear in a bacterial culture in which the possibility of introduction of bacteriophage from the outside has been completely eliminated. His conclusion is then that the

bacteriophage is a living corpuscular extracellular substance which arises from the normal living bacteria. With this as a start he elaborates his microbiotic dissociation theory.

Briefly, Hadley believes that reproduction in bacteria is by no means as simple a thing as the ordinary fission with which all are familiar. He presents the accumulated evidence suggesting that there may at times be a sexual form of reproduction in which complete living bacterial units assume forms, sometimes very minute forms, which have in the past been generally unrecognized. In support of this he mentions such phenomena as the passage of granular forms of the tubercle bacillus and of other bacteria through porcelain filters and states that with some bacteria repeated reculturing of these ultramicroscopic granular forms and probably together with certain environmental alterations in the media, sometimes causes the cultures to revert back to the original "normal" bacterial form. That bacterial multiplication is not merely a matter of simple fission is suggested by his list of accessory means of reproduction: (1) conjugation and zygospore formation followed by endospore formation, (2) gonidia formation (macrogonidia and microgonidia), (3) a process of budding which may or may not be related to the formation of gonidia, (4) propagation through the formation of simplaste aggregates (the "bacterial plasmodium" of Almquist).

Hadley's suggestion is that bacterial dissociation into its less typical forms occurs normally, particularly under specific environmental alterations. The environmental changes being kept constant there is no reason why the bacterial mutation should not persist only to be changed again when the surrounding medium becomes changed to a less propitious state.

Hadley then would attribute the change to an alteration originating within the bacterial cell. Bronfenbrenner on the other hand states that during the last four years he has attempted repeatedly by various procedures to induce the spontaneous appearance of phage in a variety of cultures known to be free from phage. So far the results have been consistently negative. He does state, however, that recently Fukuda reports having succeeded in inducing spontaneous production of phage in cultures of known purity.

There are many other hypotheses that have been suggested as explanations for the phenomenon of transmissible lysis. It has been suggested that bacteriophage resembles a growth hormone or exhibits cytotoxic properties and causes such an increase of metabolic activity in the bacteria that lysis occurs as a terminal stage of this trophic disease. Another suggestion is that with the abnormally rapid rate of bacterial growth the amount of proteolytic enzyme set free by the bacteria is so great that the supply of protein is exhausted and the ferments attack the bacteria themselves.

Bail's hypothesis closely resembles that of Wollmann. According to him, autolysis is due to the loss by the bacterial chromosomes of their anabolic function. The cells with imperfect chromosomes possessing only the function of catabolism undergo dissolution. The imperfect chromosomes are thereby set free and are capable of initiating similar mutation in other bacteria.

There are yet other theories which have few or no adherents other than their originators

In summarizing the evidence pro and con, Hadley, who is not an advocate of d'Herelle's theory, writes as follows "Bordet stated two years or more ago that d'Herelle would soon be left as the sole supporter of his virus theory. This is undoubtedly true for it is seldom now that one encounters further reports of a sustaining nature. In this regard, however, it is a curious and unfortunate circumstance that the loss of prestige of d'Herelle's view has not followed the actual disproof of any of his splendid array of facts nor has it followed the setting up of counter proof. It seems to have been determined largely by that poorly defined and scarcely scientific feeling of 'general improbability' ". Elsewhere he says, "At present it must be frankly admitted that d'Herelle's theory is the one that explains most satisfactorily the greatest number of observed facts. Moreover, the theory is so strongly fortified that it can never be disproved by resort to juggling and to reinterpreting the facts that he has presented for he has contributed the only possible interpretation of this body of facts as they now stand. In other words the disproof of d'Herelle's view as also of the theory of Bordet and Cnka depends entirely on the accumulation and analysis of new facts "

Bronfenbrenner states, "Although facts concerning the phenomenon of bacteriophagy leave an impression that the active agent of transmissible lysis is a bacterial product, the reports concerning the successful production of phage from bacteria without the intermediary of other biologic materials have thus far not been entirely convincing "

At the present time any survey of the field of hypotheses explaining transmissible lysis must be presented from an agnostic point of view. We know that the phenomenon exists but so far we possess no invulnerable theory regarding the mechanism of its production

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—W T V

Erratum

In the article by Edward Hollander, entitled *Studies in Biliary Tract Disease*, June issue, page 863, seventh line, "30 c c of 25 per cent magnesium sulphate" should read "30 c c of 33 per cent "

In the sixth line of the legend on page 865, "30 c c of 25 per cent magnesium sulphate" should read "30 c c of 33 per cent "

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CLINICAL AND EXPERIMENTAL --- ALLERGY

THE VALUE OF PHOSPHORUS AND CALCIUM IN ASTHMA HAY FEVER AND ALLIED DISEASES*

BY ALEXANDER STERLING M.D., PHILADELPHIA PA

FOR the last twenty years theories of animal experimental anaphylaxis have been advanced by different investigators. The literature up to date is full of controversies as to the real nature responsible for anaphylaxis especially the interpretation of its analogous occurrence in the human. In the latter it makes its appearance in different clinical entities known as allergic diseases for instance bronchial asthma hay fever, angioneurotic edema, and certain skin diseases for example serum disease, urticaria erythema and eczema. Others include certain gastrointestinal disorders, some forms of cystitis, arthritis, etc.

In other words anaphylaxis is produced in animals as a result of experimentation and it is analogous to the so called allergic manifestations as observed in clinical medicine. The anaphylactic phenomena are considered acquired or induced hypersensitiveness while the above mentioned allergic diseases are thought to be due to an inherited or congenital susceptibility to hypersensitiveness.

One of the most important facts of observation in allergy is a familial tendency, its occurrence in different forms in members of the same family.

In sensitive individuals we can produce at will asthma hay fever eczema etc. by direct contact by a subcutaneous injection by applying to a scratch on the forearm or to the mucous membrane or by oral administration of any of the offending proteins.

We can only state the facts which we observe from our experience every day, but cannot explain how sensitization does take place possibly in utero.

From the Asthma and Hay Fever Department Samaritan Hospital Temple University.
Read before the American Association for the Study of Allergy June 1 1928.

through maternal or paternal inheritance. Neither can one explain why one is sensitive to animal epidermals, another to pollen and still another to food proteins, etc.

Neither can one explain with any satisfaction why the sensitivity in some patients is expressed by respiratory symptoms with the production of bronchial asthma, in others, though sensitive to the same protein, it takes the form of hay fever, angioneurotic edema, or any other form of an allergic disease.

Evidently, in addition to hypersensitiveness to protein poisons, there exists a colloidal imbalance or a chemical difference in the plasma of the cells and in the serum of the sensitized individual which not only makes him sensitive but subjects him to select sensitivity resulting in different forms of allergic diseases.

Several of the above mentioned allergies may appear in a single individual at different periods of his life, such as, eczema in early infancy, bronchitis, urticaria in youth, asthma and hay fever later in life.

Some observers explain that the occurrence of different clinical forms of allergy is due to a local deficiency in the cellular structures of the parts involved. However, the introduction of sensitivity and its recognition as an etiologic factor in asthma, hay fever, etc., is one of the most important events in annals of clinical medicine. When the offending proteins are removed, the cure or the relief obtained in 85 per cent of allergic individuals is as spectacular as the results which follow the administration of diphtheria antitoxin.

The allergy in seasonal hay fever patients can be demonstrated in 95 per cent. In perennial rhinitis patients, we get only about 25 per cent demonstrable sensitivity. In bronchial asthma we find 35 per cent to 40 per cent, other investigators invariably report from 55 per cent to 65 per cent.

In seasonal hay fever, we can obtain from 90 per cent to 95 per cent of relief. In sensitive asthmatics from 80 per cent to 85 per cent, provided that sensitivity is properly established and desensitization carefully carried out.

It was not with a feeling of disappointment when we reported to the American Society for the Study of Allergy our difficulties in desensitization.¹ It was rather to stimulate further research how to benefit the asthmatic.

A good preparation is the most important prerequisite in the proper diagnosis of sensitivity. It matters little whether one uses the dermal or the intradermal method. Many of the proteins on the market obtained for testing purposes are poor, and not enough of desensitizing material in the so-called treatment sets for hay fever.

We must not forget the fact that occasionally a patient may still be positive to something for which he was *not tested*, although covering all the routine tests on the market. For illustration, a Philadelphia druggist, an asthmatic of nine years standing, was tested and treated without any improvement. Some one advised him to go to California. He spent about eight months in Los Angeles. He was sick there just the same. When he came under my care, he was tested for pollen, animal epidermals, and foods, but he did not give any reaction. On further questioning, whether any medicine in his drug

store was responsible for his illness, he suggested that he felt worse when he compounded carroid or papaine

On testing with the latter two proteins, he gave the largest reaction ever seen, a wheal the size of a silver dollar with marked pseudopods extending for many more inches. He is well since he does not handle carroid and papaine in his drug store

As a general rule, except those who work in chemical industries when a patient passes through the routine testing and does not show any reaction we consider him a nonsensitive case. Others insist that one may still be sensitive to some of the proteins tested even though the tests at the time did not show any reaction

From 55 per cent to 65 per cent of asthmatics 5 per cent of seasonal hay fever and 70 per cent to 75 per cent of perennial rhinitis do not show any protein hypersensitiveness on testing. They are usually considered to be due to inflammatory disturbances in the chest proper or to follow upper respiratory infection such as sinus involvement or any other foci of infection teeth, tonsils etc. When our patients fail to improve on surgical and medical treatment for the upper respiratory infection we look for other etiologic factors for instance, metabolic and glandular deficiencies, chronic diseases, such as tuberculosis, cardiorenal syphilis, emphysema, etc.

Drugs, intravenous medication, stool and autogenous vaccines prepared by the Cohen Heist or any other method, are used more or less empirically. We have related our experience with surgery of the nose and throat in asthmatics.² We can emphasize again the advisability for the surgeon to make a complete allergic study as well as the status of the blood calcium and phosphorus, before operative work is undertaken. Several weeks ago I had the opportunity to study a case with Dr. Nusbaum which confirms our previous observations.

S. J., twenty-two years, an undernourished and pale girl troubled for eight years with nasal obstruction, marked sneezing and rhinitis has severe coughing spells every three or four weeks lasting from three to five days occasionally wheezings. She is more or less well in the summer months of June, July, and August, worse in October and November. Two and a half years ago she had a submucous resection by another surgeon followed by bleeding rather severe for fifteen days until calcium chloride was given intravenously. She was very much worse after the operation, nasal obstruction more severe with constant sneezing and rhinitis. On testing she gave a reaction to duck feathers plus 2 and sheep wool plus 1. Her blood calcium 8.7 mg per 100 cc of blood blood phosphorus of 2.2 mg. I wonder if a surgeon is justified in operating in the presence of these clinical findings.

Surgical intervention and vaccine treatment cures and relieves many but not as many as the apparent indication for their use. However we are compelled to resort to them on many occasions. What else have we to offer our suffering patients in the absence of sensitivity or when desensitization fails to produce desired results?

TABLE I
BLOOD CALCIUM AND PHOSPHORUS IN ASTHMATICS

NAME	AGE ASTHMA BEGAN	PRESENT AGE	AL LFRGX	CALCIUM	PHOSPHORUS	CALCIUM PHOSPHORUS PRODUCT	CLINIC	RECORD
J Mc (M)	2½ yr	11 yr	Yes	100 mg	D		SH	Disp
M B (F)	7 "	10 "	Yes	100 "	D		"	"
N F (M)	7 mo	9 mo	No	100 "	115 mg	1150 mg	"	"
J M (M)	6 yr	24 yr	Yes	100 "	10 "	110 "	"	"
M Mc G (F)	32 "	35 "	Yes	80 "	D		"	"
J B (M)	6 mo	6 "	No	70 "	11 "	77 "	"	"
B T (M)	40 yr	60 "	No	110 "	22 "	242 "	"	"
H K (M)	37 "	39 "	No	80 "	40 "	320 "	NL	"
B M (F)	20 "	12 "	No	130 "	15 "	195 "	"	"
J S (M)	3 "	6 "	No	115 "	12 "	138 "	"	"
C C (F)	37 "	40 "	No	115 "	15 "	1725 "	"	"
S C (F)	6 "	21 "	No	105 "	30 "	315 "	"	"
Y C (F)	47 "	48 "	No	120 "	27 "	324 "	PC	"
F G (F)	24 "	32 "	?	90 "	154 "	1386 "	"	"
T A (M)	43 "	44 "	Yes	120 "	25 "	300 "	"	Dr R r
S G (M)	44 yr	47 yr	No	100 mg	20 mg	200 mg	NL	Disp
I R (M)	57 "	65 "	No	130 "	05 "	65 "	"	"
B F (M)	37 "	39 "	No	110 "	10 "	110 "	"	"
S T (M)	22 "	33 "	No	110 "	10 "	110 "	"	"
D O (M)	21 "	23 "	No	100 "	07 "	70 "	"	"
B C (M)	28 "	34 "	No	105 "	D		SH	"
C C (F)	40 "	42 "	No	120 "	D		PC	"
R W (M)	38 "	39 "	Yes	80 "	34 "	272 "	"	"
S P (F)	38 "	40 "	No	90 "	D		SH	"
I R (F)	46 "	52 "	No	110 "	29 "	319 "	NL	"
H W (M)	10 "	12 "	Yes	100 "	D		SH	Ward
Y E (F)	39 "	50 "	No	100 "	29 "	290 "	NL	Disp
L D (F)	21 yr	23 yr	?	60 mg	24 mg	144 mg	SH	Disp
G W (M)	32 "	33 "	Yes	105 "	D		PC	Dr B v
S T (M)	4 "	9 "	?	100 "	10 "	100 "	SH	Disp
B S (F)	20 "	30 "	Yes	100 "	20 "	200 "	PC	Dr S n
J B (M)	60 "	66 "	No	115 "	45 "	5175 "	NL	Disp
J H (M)	58 "	60 "	No	110 "	31 "	341 "	"	"
J P (M)	4 "	7 "	No	90 "	30 "	270 "	PC	"
B M (M)	34 "	42 "	Yes	100 "	05 "	50 "	"	"
I L (F)	20 "	30 "	No	90 "	33 "	297 "	NL	"
J S (M)	5 "	14 "	Yes	100 "	D		PC	"
E P (F)	50 "	52 "	No	106 "	D		SH	Ward
J F (M)	48 "	58 "	No	110 "	12 "	132 "	PC	Dr G v
M K (M)	32 yr	40 yr	?	130 mg	11 mg	143 mg	NL	Disp
D K (M)	31 "	38 "	?	80 "	20 "	160 "	"	"
G D (F)	22 "	23 "	No	80 "	34 "	272 "	PC	Dr G g
S G (M)	2 "	5 "	Yes	95 "	38 "	3610 "	"	Dr E k
M L (M)	26 "	27 "	No	92 "	38 "	3496 "	"	Dr S d
T R (M)	4 "	13 "	No	100 "	22 "	220 "	"	Dr M l
A M (M)	28 "	30 "	No	125 "	D		SH	Ward
F A (M)	34 "	35 "	Yes	116 "	21 "	2436 "	"	Disp
B H (M)	48 "	57 "	No	96 "	29 "	2784 "	"	"
F R (M)	39 "	41 "	No	101 "	35 "	3535 "	"	"
J W (M)	5 "	8 "	Yes	126 "	28 "	3528 "	PC	Dr W e
B C (F)	31 "	37 "	No	110 "	24 "	264 "	SH	Disp
H Mc (F)	34 "	38 "	No	60 "	11 "	66 "	"	"
E W (F)	38 "	41 "	No	90 "	26 "	234 "	NL	"

D = Deficient below 0.5 mg

SH = Samaritan Hospital

NL = Northern Liberties Hospital

PC = Private Case

TABLE II
BLOOD CALCIUM AND PHOSPHORUS IN HAY FEVER
OR POLLEN POSITIVE SEASONAL CASES

NAME	AGE HAY FEVER BEGAN	PRESENT AGE	AL LERGY	CALCIUM	PHOSPHORUS	CALCIUM PHOSPHORUS PRODUCT	CLINIC	RECORD
M H (M)	16 yr	23 yr	Yes	80 mg	D		SH	Disp
J H (M)	12	28	"	100	D		PC	Dr M rg
H R (M)	11 "	14 "	"	100 "	10 mg	100 mg	"	Dr H nd
I N (M)	39 "	44 "	"	90 "	24 "	216	N L	Disp
H W (M)	30 "	38 "	"	75 "	D		PC	Dr M ll
Dr W (M)	13 "	20 "	"	00 "	D		SH	Disp
E K (M)	7 "	10 "	"	105 "	D		PC	Dr K s
J C (M)	29 "	36 "	"	100 "	D		"	Dr G o
S R (F)	28 "	33 "	"	110 "	15	165	"	Dr E k
M S (F)	37 "	42 "	"	113 "	D		SH	Disp
K T (M)	7 yr	13 yr	Yes	110 mg	D		SH	Disp
B G (F)	13 "	15 "	"	120	42 mg	504 mg	N L	"
S Mc (M)	15 "	47 "	"	109 "	11	1199	PC	Dr M ll
D W (M)	7 "	26 "	"	113	11	1243	SH	Disp
B S (M)	27	32	"	110	14	154	"	"
J C (M)	33 "	36 "	"	110 "	15	165	"	"
E S (F)	4	5	"	100	37	370	PC	Dr G s
R O (F)	5 "	6 "	"	120	24	288	SH	Disp
A N (F)	0	36 "	"	124	34	4216	"	"
S F (F)	3 "	"	"	112	31	3472	"	"

TABLE III
BLOOD CALCIUM AND PHOSPHORUS IN PERENNIAL RHINITIS

NAME	AGE RHINITIS BEGAN	PRESENT AGE	AL LERGY	CALCIUM	PHOSPHORUS	CALCIUM PHOSPHORUS PRODUCT	CLINIC	RECORD
R R (F)	29 yr	35 yr	No	110 mg	33 mg	363 mg	N L	Disp
S G (M)	24	34	"	104	28	2912	PC	"
M C (F)	38	45	"	50	05	25	SH	"
B M (M)	15 "	17 "	"	110	08	88	N L	"
L A (M)	46	57	"	120	24	288	"	"
S B (M)	28	30	Yes	100	D		PC	Dr C n
F H (F)	77	39	No	90	35	315	N L	Disp
F S (F)	20	21	"	112	31	3472	PC	"
M K (F)	30	37	"	110	37	365	N L	"
I C (M)	1	3	"	90	20	180	SH	"
J G (M)	12	13	"	100	20	200	"	"
S J (F)	14 "	22 "	Yes	87	22	1914	PC	Dr N m

For many years it has been observed that allergic patients improve on the administration of calcium salts. On account of this the theory of calcium deficiency in asthmatics was established. Study was made on patients from the Asthma and Hay Fever Department of the Samaritan Hospital from the Chest and Asthma Clinic of the Northern Liberties Hospital and on a number of private patients.

In this series a study of 85 cases of blood calcium, it shows a calcium deficiency in only a small per cent still many patients improve on calcium. In this very same group we have made a study of blood phosphorus and we were surprised to find a very striking phosphorus deficiency as compared with normal or high blood calcium. We see by the accompanying charts, that only twenty three patients had a low blood calcium of 95 mg or lower while the rest, sixty two patients, had a normal and a high normal blood calcium, rang

TABLE IV
BLOOD PHOSPHORUS IN 85 ALLERGIC PATIENTS

a Bronchial asthma	53	35 male	18 female
b Hay fever	20	13 male	7 female
c Perennial rhinitis	12	6 male	6 female
Blood phosphorus from 0.1 to 1.0 inclusive			30 patients
a Bronchial asthma		18	
b Hay fever		9	
c Perennial rhinitis		3	
Blood phosphorus from 1.1 to 3.0 inclusive			35 patients
a Bronchial asthma		26	
b Hay fever		9	
c Perennial rhinitis		5	
Blood phosphorus from 3.1 to 4.5 inclusive			17 patients
a Bronchial asthma		9	
b Hay fever		4	
c Perennial rhinitis		4	

TABLE V
BLOOD CALCIUM IN 85 ALLERGIC PATIENTS

a Bronchial asthma	53	35 male	18 female
b Hay fever	20	13 male	7 female
c Perennial rhinitis	12	6 male	6 female
Blood calcium from 5.0 to 7.0 inclusive			4 patients
a Bronchial asthma		3	
b Perennial rhinitis		1	
Blood calcium from 7.1 to 9.5 inclusive			19 patients
a Bronchial asthma		12	
b Hay fever		4	
c Perennial rhinitis		3	
Blood calcium from 9.6 to 13 inclusive			62 patients
a Bronchial asthma		38	
b Hay fever		16	
c Perennial rhinitis		8	

ing from 9.6 mg to 13 mg per 100 cc of blood. On the other hand, sixty-eight patients show a phosphorus deficiency and only seventeen have a normal blood phosphorus from 3.0 to 4.5 mg per 100 cc of blood. We have observed that many patients with a low blood phosphorus make a spectacular improvement of phosphorus, especially when used in addition to any other standard form of treatment.

For illustration a boy, ten years old, was suffering from asthma since childhood. He was undernourished, pale, and perspiring freely on the slightest exertion. While awaiting the regular routine study, he was put on phosphorus. He made an immediate improvement and was practically free from asthma ever since, which is for the past eleven months. He gained twenty to twenty-five pounds in the first three or four months of treatment. The blood calcium was 10 mg, while his blood phosphorus was too small to be determined quantitatively. Two months later his phosphorus was 2 mg, six months

TABLE VI
BLOOD CALCIUM PHOSPHORUS PRODUCT IN 85 ALLERGIC PATIENTS

a Bronchial asthma	33	35 male	18 female
b Hay fever	20	10 male	7 female
c Perennial rhinitis	12	6 male	6 female
<hr/>			
Blood calcium phosphorus product from 0.1 to 20 inclusive	31 patients		
a Bronchial asthma	31		
b Hay fever	14		
c Perennial rhinitis	6		
<hr/>			
Blood calcium phosphorus product from 20.1 to 30 inclusive	16 patients		
a Bronchial asthma	12		
b Hay fever	2		
c Perennial rhinitis	2		
<hr/>			
Blood calcium phosphorus product from 30.1 and above	18 patients		
a Bronchial asthma	10		
b Hay fever	4		
c Perennial rhinitis	4		

later 4.4 mg. This was the first case that impressed us with the study of phosphorus and the value of its use in asthmatics.

An illustration of the value of phosphorus in hay fever. A young man twenty years of age, a dental intern, has had summer hay fever for seven years. While he was in Philadelphia working in his hospital he was comparatively comfortable but at week ends when he visited his parents on the farm, he was having severe attacks of hay fever. He was tested found strongly positive to summer grasses orchard grass June grass timothy and sweet Vernal, each plus four. The blood calcium was 9 mg. phosphorus too small to be determined quantitatively. He was put on phosphorus and at the same time he was requested to abstain from any other medication and to report his experience when visiting the farm at week ends. On his return he reported he was practically free from any hay fever attacks.

Our studies show that 66 per cent of hypersensitive and 34 per cent of the nonsensitive have a low blood phosphorus. Of the 23 cases who had a low calcium, only 7 or 30 per cent were sensitive.

All the patients whether sensitiveness can be proved by testing or not, are put on phosphorus with variable results. In most cases the results are good.

Many of us have witnessed the asthmatic in his paroxysms, the labored breathing the fatigue of the muscles of respiration as well as the exhaustion of the entire body. Among the students of physiologic chemistry it is known that exercise increases the excretion of phosphates. In the so called 'fatigue substances' of muscle among other excretions the most important is potassium dihydrogen phosphate. Therefore, the state of muscle fatigue in the asthmatic may possibly be responsible for the instability or phosphorus deficiency, as observed in this series of cases.

Since our administration of phosphorus in addition to desensitization in hay fever patients, the results have been almost 100 per cent freedom from symptoms.

Young asthmatics who perspire easily on the slightest exertion, get colds often do not perspire easily and are not subject to colds after a few weeks on phosphorus

Calcium is beneficial in most hypersensitive cases, whether they show a low blood calcium or not. It produces a marked improvement in seasonal hay fever, in some cases of perennial rhinitis and in bronchial asthma, it stops the leaking sensitive mucous membrane of the upper respiratory system. Both calcium and phosphorus help to control the hyperexcitability of the nervous system.

The interrelationship between calcium and phosphorus metabolism has been recognized for some time, which is expressed by Howland and others "that an excess of calcium impairs phosphorus retention, excess in phosphorus is unfavorable to calcium absorption." Clinically we find calcium and phosphorus both low in some individuals, on the other hand, calcium and phosphorus both high in others.

COMMENT

Hay fever patients and pollen asthmatics under our care last fall (1927) had absolute freedom from symptoms. The results were probably due to our better knowledge in desensitization, but we think that it was due to the addition of phosphorus and calcium medication.

The mucous membrane is constantly bathed in the (circulating) blood serum which probably contains a great many defensive bodies or enzymes. The activity of the latter depends upon many unknown substances called activators. The only one known is phosphate.

In normal individuals it is quite possible that inhalants, food proteins, etc., are digested or converted by these defensive bodies when activated on by many substances as yet unknown (the only one known is phosphate). This process probably renders the protein absolutely harmless when it gets in contact with the mucous membrane of the respiratory or gastrointestinal system.

On the other hand, in the allergic patients, the inhalants, food and bacterial proteins are not being activated on, therefore, they are not destroyed or converted by the defensive body substances. Consequently the above mentioned proteins act as irritants, producing asthma, hay fever, etc.

The allergies as shown by our charts are deficient in phosphorus, some in calcium and improve when the latter two are added to the treatment. F. J. Novak,¹ J. I. and A. R. Hollander,² R. Sonnenschein and S. J. Pearlman,³ F. M. Pottenger,⁴ and Graftis T. Brown and Oscar B. Hunter⁷ found calcium deficiencies in allergies and reported improvement in the majority of these patients upon the addition of calcium.

Therefore, are we not justified to infer that phosphorus and calcium are probably playing the rôle of activators for the defensive or enzymic activity in our blood stream, and help in the proper conversion or rendering the inhalants, bacterial and food proteins harmless when they reach the mucous membrane?

The relation of physiologic chemistry to the problem of allergy requires further investigation, with reference to phosphorus and calcium metabolism and other blood minerals, especially the possibility of the presence of enzymic activity in the blood serum

CONCLUSION

While we cannot draw positive conclusions from a study of the above number of cases calcium and phosphorus are found of great benefit in the treatment of allergic diseases. This mineral deficiency in asthmatics, hay fever and allied diseases whether sensitivity can be proved or not, may be the missing link which makes the allergic differ from the normal. The addition of calcium phosphorus, and other minerals, for instance iron, sodium and potassium salts are possibly responsible for the favorable results in this series of cases.

I wish to acknowledge my appreciation to Dr Wm Egbert Robertson for some suggestions in the construction of this paper, and to Dr Clark, to Dr Ginzburg, pathologists to the Samaritan and the Northern Liberties Hospitals respectively, for the laboratory work in the blood calcium and phosphorus

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A SIMPLE CHART BASED ON THE STUDY OF FOUR HUNDRED CASES WHICH ILLUSTRATES OUR PRESENT KNOWLEDGE OF ALLERGY*

BY MILTON B. COHEN, M.D., CLEVELAND, OHIO

ALTHOUGH the conception of asthma as a manifestation of human hypersensitiveness is accepted by most clinicians, in many of the individuals suffering from this disease it is still quite impossible to determine the active cause which sets up the reaction. And in some patients, even when the exciting cause is known, it is impossible either to remove it sufficiently from the patient's environment or to raise his tolerance to it to a sufficiently high level to prevent the occurrence of symptoms. Also, in a fairly large number of patients one is sure to find several in whom attacks seem to be brought on by purely nonspecific causes, such as temperature changes, nervous influences, mechanical factors, and certain infections. Thus the protean nature of asthma and the difficulties in evaluating the various factors in its etiology and treatment have led to a great deal of confusion, not only in the minds of the average physicians, but also in the minds of those working intensively with these patients. Each worker has been inclined to look at the problem from the angle of his own particular specialty. Thus we have nasal, infectious, toxemic, and neurogenic theories as well as the hypersensitive theory.

There seemed to be a need for some conception of allergy which would be scientifically correct for the present state of our knowledge and would at the same time correlate the known facts and observations. Accordingly, the accompanying chart was prepared. It suffices to classify and explain any and all symptoms occurring in any patient, and points out the principles necessary for their relief. It is interpreted as follows:

In the center, dividing it into two parts, is a long block labeled *Tolerance*. While all allergic patients will react to the substances producing their attacks, the tolerance is different in each, and may vary in the same individual from time to time.¹

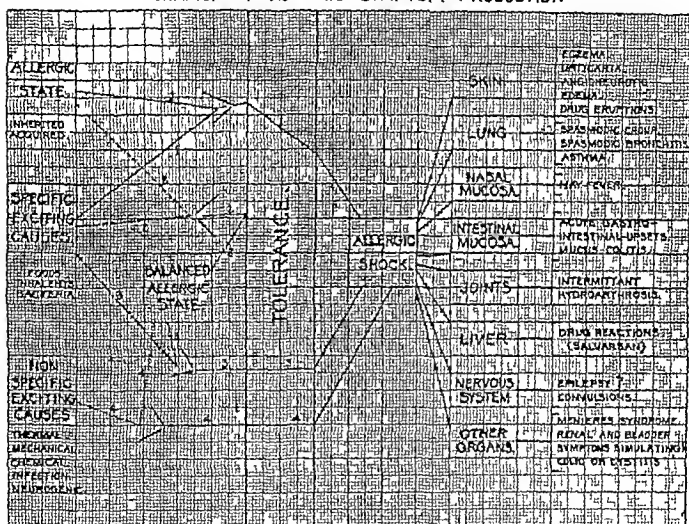
Reactions occurring on the left side of the tolerance block are symptomless, those passing beyond it to the right side of the chart are all productive of symptoms.

In the upper left-hand corner of the chart is a block labeled *The Allergic State*. This represents the hypersensitive state; patients in this state have been sensitized to some specific substance. Despite the evidence of the hereditary nature of this condition, there is evidence to believe that only the predisposition, or facility to be easily sensitized, is inherited, and that the allergic state is produced only after active sensitization by contact with the specific substance or substances after birth.^{2, 3}

*From the Medical Service and the Allergy Clinic of Mt. Sinai Hospital.

Below the block representing the allergic state is a block called the *Specific Exciting Causes*. These may be foods, inbilitant substances such as animal epidermals, products of industry, molds dusts, and bacteria. When a patient allergic to some specific cause receives a dose greater than his tolerance, allergic shock occurs, as can be seen by following Lane 1. The site of the allergic reaction will depend in each case on the relative sensitivity of the different end organs of the body. These vary in their sensitivity so that in one individual there may result hay fever in another asthma, in another skin eruptions in another gastrointestinal symptoms, or there may be combinations of these or any other symptoms as indicated in the blocks at the extreme right of the chart. This is the mechanism of the frank allergic reaction.

MECHANISM OF ALLERGIC SYMPTOM PRODUCTION



Frequently, however patients who are exposed to specific allergens constantly react periodically despite their daily exposure. During their free period these patients are in the *Balanced Allergic State*, a term coined by Dr W T Vaughan⁴. This state may be produced either by a temporary raising of the level of tolerance (desensitization) or by variations in the dose of the specific substance or substances. As may be seen by following Lane 2 when a patient in the allergic state receives a dose of specific allergen less than his tolerance no symptoms result but he is placed in the *Balanced Allergic State*. This state is particularly labile. While in it an extra dose of some specific allergen or a reduction in tolerance may allow it to be exceeded, with a resultant allergic reaction.

At the bottom left-hand corner of the chart there is a block named *Nonspecific Exciting Causes*. Their number is legion, but they may be grouped under the headings, Thermal, Mechanical, Chemical, Infectious, Neurogenic. These have caused endless confusion in the study of allergic patients as they have frequently been considered by both patients and physicians as major causes. A very simple and common example is presented by the infant with eczema. The pediatrician attempts to adjust the diet, the dermatologist keeps irritants away from the skin, the allergist tries to remove from the patients the specific allergen. In some patients either method is sufficient, in others a combination is required. Depending on the method used to produce the healing, a different physiologic pathology is ascribed to each case. In the case relieved by ointments, etc., it is probable that the infant, in the *Balanced Allergic State*, has had symptoms produced by the additional mechanical irritation of soap, water, and scratching. The removal of these irritants afforded an opportunity for the return to the symptom-free *Balanced Allergic State*.

Another characteristic example is presented by the case of J. G., aged thirteen years, who had had attacks of asthma occurring every two or three weeks for six years. When first studied he was found to be feather sensitive, but attacks could be induced during the free interval by breathing in cold air while the chest was rubbed with ice. Feathers were removed from the home, and all attacks ceased and have not resumed for two years. One month after beginning residence in a feather-free house, an attempt was made to induce an attack by means of cold, as described above. Despite repeated exposures, no reaction occurred. In this boy, feathers induced the *Balanced Allergic State* which, during the intervals between attacks, could be activated into allergic shock with asthma by means of a dose of a nonspecific exciting cause, cold.

The mechanism described by which a patient passes from the *Balanced Allergic State* to allergic shock, can be followed by means of Lines 3 and 4.

From a study of this chart it will be seen at once that the treatment of allergy may be accomplished in one of two ways:

1. Remove the allergic state.

2. Reduce the doses of the specific and nonspecific causes below the patient's threshold of reaction so as to maintain him in the *Balanced Allergic State*.

The first method is impractical in most cases. In the first place, the inherited facility to acquire sensitivity is a fixed characteristic and cannot be changed. Secondly, it is extremely difficult to demonstrate beyond reasonable doubt the exact specific allergens responsible for the allergic symptoms, and it is not yet possible to obtain these in pure form. Also, patients are usually sensitive to more than one allergen. Attempts to raise the tolerance to a level comparable with that of nonsensitive individuals have been partially successful in cases with pollen disease, namely, hay fever and its accompanying asthma, but even in these the tolerance is lasting in only a small percentage. Until we have better knowledge of the exact nature of

specific allergens, and of the physical chemistry of the allergic response, removal of the allergic state is impossible except in rare instances

The second method of treatment while sufficiently difficult to tax the ingenuity of the physician to the utmost, is nevertheless practical and affords relief for the large majority of patients. It is a broad clinical problem requiring the use of various special methods of study plus good common medical sense. The history of the attacks, their periodicity, their relation to specific exposures, their disappearance on change of residence or of climate all afford clues. Skin tests with stock food and inhalant substances and with extracts of dust from the environment afford other clues. Bacteriologic study of the respiratory flora, and skin tests with these organisms give additional clues. Search for foci of infection and for other intrinsic pathology should be made. All clues must then be subjected to careful clinical experimentation varying one factor at a time, to determine their relationship to the patient's symptoms.

A simple outline for the study of a case of asthma is the following.

- 1 Remove the patient for a period of two weeks to an environment which is free of all inhalant substances. Such a condition can be produced in any room by means of a thorough renovating covering the mattress and pillows with impervious material, and the installation of one of the electrically operated mechanical filters now on the market.

- 2 During the stay in the dust free room perform routine and special skin tests and study the effects of heat, cold and other physical factors.

- 3 If the patient's symptoms disappear while in the dust free room attempt to correlate the symptoms with some inhalant factors in his home or working environment by actual exposure to these substances and the production of an attack.

- 4 Rearrange the home environment in accordance with the information gained by the above study either by removing the offending articles of furniture, or the production of a dust free room at home for the part time use of the patient.

- 5 Arrange the diet in accordance with information obtained by the history and tests.

- 6 For the patients who fail to get relief by the use of these methods attempts at specific desensitization with the bacteria to which they are sensitive should be tried.

- 7 Nonspecific desensitization may be of use in rare instances and for symptomatic relief ephedrin and adrenalin are helpful.

SUMMARY

Based on the study of four hundred patients with asthma a chart has been prepared which correlates our present knowledge of allergy, explains the mechanism of symptom production, and indicates the principles to be followed in its treatment.

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10616 EUCLID AVENUE

SUBNORMAL TEMPERATURE IN THE PERENNIAL ASTHMATIC PATIENT*

By WILLIAM W DUKK, M.D., KANSAS CITY, MO

THE temperature of a patient with asthma which is caused by sensitiveness to material things, such as pollen or hair or food, I have not found to be very interesting. There is a group, however, which does not react to anything except, as far as you can determine by history, to the effect of heat or cold or to the combined effect of the two. These patients have very severe types of asthma. Attacks can be produced by applying heat or cold in a given way and relief obtained by applying it in the reverse way. I gave a paper on this subject some time ago. I think this type of asthma is just as definite a disease as pollen disease.

I believe it is due to some damage which has been done to the heat regulating mechanism. Physicians in this line ought to recognize what a complicated mechanism the heat regulating mechanism must be. One may go to the equator and then to the north pole, and his body temperature will stay the same if he is a normal individual. Temperature is regulated largely through the constriction or dilation of the vessels and capillaries in the skin or nasal tract and bronchial tubes. Cooling takes place by radiation of the heat of the body through the skin, by evaporation of the moisture on the skin, and especially by evaporation in the bronchial tubes. Where there is little moisture on the skin and very little radiation on account of the heat in the air, cooling from the skin is not maintained and temperature is regulated almost entirely by the bronchial tree. If there were to be trouble in this heat regulating mechanism so that its work is not well done, there would be all kinds of abnormal reactions, it seems to me, in the bronchial tubes, nasal membrane, and skin. This mechanism may also partly control oxidation in the body for the generation of heat.

There are two types of individuals—one the normal, while the other reacts in a peculiar way with the production of an overstimulation in the autonomic nervous system.

In one the reaction is explosive, and rather purposeless, and in the other the reaction is inflammatory. People who are sensitive to heat and cold fall

*Read before the American Association for the Study of Allergy Minneapolis Minn June 11 1928

into several types. The ones who are sensitive to heat alone have their illness in the summer time. The ones who are sensitive to cold alone, that is lowering of the body temperature have their trouble in the dead of winter. There may be combined cases which may have their attacks depending upon the temperature of the outside air and their body heat, that is the nasal membrane may be sensitive to cold air local cold when their body is hot others may be sensitive to warm air when their bodies are cold, and others are sensitive to breathing of warm air or moisture when their bodies are cold. These two types are rather common and have very severe illnesses.

Another type is the one which reacts to cold air after exposure to heat or heat after exposure to cold. Patients of this type who react to cold after exposure to heat, may exercise and get hot in the summer time and then when they sit down in the cool of a shade tree they will react with asthma. The reverse type will in the winter time after they have been out and chilled by exposure to a cold temperature come into the house warm up and then have an attack of asthma. In certain cases this acts seasonally, that is a person after exposure to the cold of winter will react when the warm days of spring come along. Finally, they gain tolerance for heat and will be all right in the summer. The reverse type, the cold sensitive patient will react in the fall, that is after they have been exposed to the heat of summer. They react to the first cold days of winter. These cases are as definite as can be. Reactions are not confined to the respiratory tract but give all the manifestations of allergy, skin manifestations, and all.

In the regulation of body heat the nasal mucous membrane, the bronchial mucous membrane and the skin play an important part. If the patients belong to the allergy type of individual their symptoms may be indistinguishable from hay fever if they belong to the nonallergic type the reaction comes out in a different manner, more chronic and inflammatory. The skin manifestation sometimes appear in the form of an eczema the ugliest you have ever seen.

In one case twelve hours after exposure to heat whether the heat of the air or exercise, the patient would develop an attack of asthma. If he kept himself quiet and cool possibly his reactions would not be quite so great.

Heat sensitive patients usually run subnormal temperatures and usually react most severely when the temperature is inclined to be lowest.

The most extreme example I have had was a man who once had a morning temperature of 92° . It was a rule to have $94\frac{1}{2}^{\circ}$ in the morning and 96° in the afternoon. The slightest heat would make him break out. He did not sweat. His skin was as dry as paper.

Several times we have tried intravenous peptone therapy and calcium. The temperature when stabilized very often is followed by complete relief. I think the relief which comes from peptone and calcium, and other apparent nonspecific reagents lies in modifying the action of the heat regulating mechanism. Infectious disease associated with fever will almost invariably relieve these patients for the time being. It may be that it picks up the heat regulating mechanism.

If heat sensitive patients have tonsillitis, erysipelas, or pneumonia, or as in one case, a lung abscess, they are temporarily relieved. When the temperatures come back to normal, they are more stabilized than before, and I think they may be cured of asthma for from a month to several years.

CHRONIC USE OF ADRENALIN IN THE TREATMENT OF ASTHMA*

By WILLIAM W. DUKE, M.D., KANSAS CITY, MO

MY CHIEF reason for giving a paper on the chronic use of adrenalin is to get some discussion on the subject, and really learn something about it, because I do not feel that we are sure, by any means, that the prolonged, continuous use of adrenalin is a good thing for a person.

Everyone who has treated much asthma for any length of time, has had patients who have been taking adrenalin—four and five doses a day—for years before they came to them. I have three patients who have taken adrenalin in stupendous doses, doses amounting to as much as four ounces in twenty-four hours, two or three in a dose every fifteen minutes for prolonged periods of time. Patients doing that are usually in an abominable state, have a tremor, feel weak, and are very often intensely pigmented. Two of my patients have looked as though they had Addison's disease. They were as brown as that.

I do not think there is any doubt in the world but that that sort of administration of adrenalin is harmful. Whether or not the intelligent daily use of adrenalin is harmful is a question. I should certainly like to hear something pro and con on that subject.

It seems to me that it is a good idea to get a person away from adrenalin if possible, simply because it is a good idea to get a person away from any unnatural drug, especially a drug that is as potent as adrenalin.

In these patients who are taking enormous doses, the dose can be reduced greatly if one thing will be borne in mind, and that is that there are two kinds of edema in allergy, and very often in a given patient both types will be present. One is the allergic edema that gives rise to bronchial constriction in the ordinary allergy case, I mean bronchial obstruction, due to allergy. The other is mechanical edema which has nothing to do with allergy, it is simply caused by the mechanical effect of air rushing up and down the bronchial tubes, the same as it will cause edema in anyone.

I have a wonderfully instructive example of this type, a patient who had a bad pollen reaction at a time when the pollen content of the air was enormous. The man had a mild reaction, he went on a trip into the country in an automobile and had an intense exposure to weeds. He began rubbing one eye and soon came back to my office with this eye enormously swollen. The other was mildly swollen. I gave adrenalin and brought the eye which

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was slightly swollen back to normal in a short time. The other eye remained swollen for a considerable period of time. If there is a swelling of the membranes in the bronchial tubes is it due to allergy or is it in part mechanical? If the former we can get relief almost always if intelligently administered. If the latter we may get partial relief but pushing the drug to enormous dosage will not give complete relief. There is little purpose in pushing adrenalin to such doses as I have seen given in an effort to accomplish the impossible.

When we have patients who have been taking adrenalin several times a day for years, how can we get the patient away from it and is it advisable? I found a very good substitute in the form of ten or fifteen grains of aspirin together with a good dose of whisky before the inception of the attack. That therapy, if it is adequately administered is just as effective as morphine and very much less dangerous than morphine. When allergic patients become addicted to morphine sometimes they seem to become sensitive to a lack of morphine. After that time it is impossible to cure the asthma or the morphinism. So morphine ought to be avoided in allergy just as rat poison would be avoided. Personally I do not use it over once a year in the case of asthma, except in patients already addicted to it. One can always get by with a large dose of whisky and aspirin. If enough whisky is given them to make them sleepy and they are made to lie down and go to sleep that is usually the end of the attack of asthma.

If this treatment is combined with other little things we do for asthma especially getting the patient away from the source of the trouble or immunizing him, the dose of adrenalin can usually be reduced to a few drops at a time, maybe once or twice a night, and as a rule it is possible to get rid of it completely.

DISCUSSION

Dr Ray M Balyeat Oklahoma City Okla—Due to the redskins in the state of Oklahoma we are either very unfortunate or fortunate that we are unable to use the combination that Dr Duke referred to that is five grains of aspirin and a swig of whisky every fifteen minutes, but since the experience of a couple of doctors who used to live in Dallas who now live in Leavenworth Kansas I feel sure that we are very fortunate for such prescriptions cannot be used in our state.

I was very much interested to know what Dr Duke was going to say about the chronic use of adrenalin in allergic cases because for several years I have been a firm believer in the use of adrenalin. We teach all patients with whom we deal how to use adrenalin because I believe that a patient who is suffering from asthma who knows how to use adrenalin will be a much more practical individual. For example if he is a business man traveling from our city to Chicago he will realize that if he should have an attack of asthma on the pullman he has a means with him that will protect him from asthma. If he be at home instead of wheezing if asthma appears any hour of the night he realizes that he has a means of relieving his attacks and consequently he will retire feeling very much better and without the worry that he would naturally have if he knew that he would have to suffer several hours or call a doctor for relief. Many patients as we all know will not call a doctor when they begin to wheeze on account of the expense or the inconvenience, and therefore will continue their wheezing and frequently produce an emphysema of the lungs which in turn means cardiac failure.

I have often stopped and wondered whether we actually did damage in allowing a patient to continue the use of adrenalin over a long period of time. It has been my

experience, however, and the experience of others that I have talked to, that so far as we can see no damage is actually done. I talked to Dr. Walker about this matter last winter. I am very anxious to know how Dr. Pottenger feels about it. Personally, I feel that there can be little damage done from a drug that is a normal constituent of the blood stream. The effect of adrenalin is evanescent, which is another reason why I believe it is harmless. Surely it is not going to have the serious effect on the asthmatic patient that the long, strenuous effect of the asthmatic spasm would have.

In regard to morphine, I have seen very few allergic people who have developed a habit of using morphine. In our work with allergic cases we prescribe it very seldom. A narcotic inspector visited my office a few days ago and asked me to give an account as to why morphine prescriptions did not appear in the drug stores in Oklahoma City written by me. I told him I thought it was not any of his business. He looked at me very squarely and said, "In the state of Oklahoma I find prescription after prescription for morphine that is marked on the back, 'advanced asthmatic,' and I just wondered what you thought of the use of morphine for asthma."

Recently there was called to my attention by a nurse patient of mine in Amarillo the fact that two or three nights ago she had given some adrenalin in her thigh and it got to itching that night and hurting to some extent, which caused her to massage the area. The massaging of the area relieved a subsequent attack of asthma that had come on. About that time, a couple of weeks afterward, I noticed in the American Medical Journal a short note by Lohenthal concerning the use of adrenalin for the relief of bronchial attacks in postoperative cases of tuberculosis. Since that time we have tried it out on quite a few true asthmatics and find that in at least 75 to 80 per cent of them, if the attack of asthma comes within eight hours after giving adrenalin, that massaging the area will relieve the attack, which I believe is going to be of considerable help from the standpoint of the use of adrenalin. It means that adrenalin doesn't have to be given again and again. So many times a doctor will go into the country and give a dose of adrenalin to the patient, and in four or five hours from that time the patient is suffering again. Such an experience will cause him many times to give morphine instead of adrenalin. If by teaching that patient to massage the area relief can be had I think it will be considerable help in the use of adrenalin for the relief of asthma.

I would like to ask Dr. Pottenger to discuss one particular thing if he will, namely, why acute infections will relieve attacks of asthma. It is notoriously true that certain types of acute infections will relieve asthma. I have seen a good many cases in which an acute appendix, or scarlet fever, or typhoid, especially typhoid, relieved the patient from attacks of asthma for several months. I have one at present who has asthma with epilepsy and I once told the mother that if he could have any severe acute infection he would probably be free from asthma and also his epilepsy. He had an attack of scarlet fever and his epilepsy was gone for a year and is just now returning. Personally, I believe the toxin which stimulates the sympathetic nerve opposes the parasympathetic nerve, thereby giving relaxation.

Dr. Bernhard Steinberg, Toledo, Ohio—The failure of adrenalin to relieve asthmatic attacks may be explained in some instances on anatomic grounds. The case I am to present illustrates this condition. The bronchioles, small and medium sized bronchi are completely occluded by thick mucus which adheres to the walls of the tubes. If the result of the action of adrenalin is to dilate the bronchi, the adherent, thick mucus will prevent that action. The patient in this case was unable to raise any bronchial secretion.

Dr. F. M. Pottenger, Monrovia, Calif.—There are one or two things which I should like to say regarding the cases that Dr. Duke speaks of with low temperatures and high temperatures.

It was rather interesting that the whole picture of vagotonia or parasympatheticotonia is a group in which anabolism is stronger than catabolism. That is characteristic of this group of individuals, and I think that is one of the reasons so many of them do have the lower temperature. They belong to the hypoglycemic type.

I was a little interested in what has been said about using insulin. I would hardly expect insulin to help the average case of asthma, particularly if it belongs to the hypoglycemic type which it usually does.

I think he points out a very interesting thing in this question of heat. When we are dealing with so many variables and so many things that interfere with our normal physiology it is hard to pick out one variation which is always active.

With regard to the point brought out by the last gentleman a few years ago I saw a little abstract of a paper by some one I have lost the name and the reference. He found that in the secretions of the asthmatic there was a substance that produces a spasm of the smooth muscles and thus agrees with what we have just mentioned and also with what we should find. With a severe case of asthma during a period of prooxysms I have given sodium iodide. Why? I want to get rid of the expectoration so if you give sodium iodide, about fifteen or twenty cc intravenously and repeat every two or three days you will find it will give tremendous relief, although it is opposed to the action of the calcium physiologically.

I raise the question of why acute infection produces relief from asthma. It cannot be answered entirely. I don't think we can point to any one thing but you will find that any acute infection acts preeminently upon the sympathetic nerves. The whole peripheral picture of an infection expresses itself nervously as the sympathetic nervous system. There is more H than OH acidity rather than alkalinity. A metabolic process is going on with the temperature raised and all the features that oppose the ordinary physiologic principles that are present in parasympatheticotonia. There is also during these periods a stimulation of the adrenals, with increased adrenalemia during infection. There is also increased stimulation of the thyroids during this period of reaction so that the whole physiologic mechanism that acts to oppose the parasympathetic mechanism is uppermost. I don't know the one thing to lay it to but it seems to me that it is a complex affair in which many of the different systems are involved.

Dr M. Murray Prishin, New York City—I have always felt that a discussion on the use and abuse of epinephrine hydrochloride in the treatment of asthma would be of timely interest. First of all it should be recalled that the physiologic action of adrenalin (1:1000) depends upon the quantity injected. Following the injection of a large dose of adrenalin (eight or more minims) the rate of the pulse is often increased thirty or more beats per minute the systolic blood pressure is frequently raised to dangerous levels the rate of respiration is increased breathing at first becomes shallow and convulsive in character accompanied by tremor, pallor, cardiac palpitation, weakness and giddiness. Following the injection of a small dose of adrenalin (two to four minims) the picture is entirely reversed. The pulse and respiration rate are lowered the depth of respiratory movement is increased and there is an absence of tremor, pallor, cardiac palpitation and weakness. The systolic blood pressure is frequently lowered ten or more millimeters of mercury. It is perfectly safe to administer two minims of adrenalin in an attack of asthma with a systolic blood pressure of 200 or more. If a patient is only partially relieved from an attack of asthma following the injection of a small dose of adrenalin a second dose administered fifteen minutes later will invariably bring about relief without causing symptoms that usually follow the injection of a single large dose of adrenalin. If this method of administering adrenalin were commonly employed I feel certain that the number of patients employing from one to two centimeters of adrenalin at one time and those with adrenalin tolerance would be considerably lessened.

It is extremely difficult to conceive of a human being escaping adrenalin tolerance after taking four ounces of the drug weekly for several years. A patient with an increased adrenalin tolerance usually gives a history of injecting the drug in progressively increasing doses. Such patients first came under my observation about nine years ago. Since then it has been my practice to break their tolerance for adrenalin by injecting eight minims each of adrenalin and extract of pituitary (obstetric strength). This combination usually causes pain at the site of the injection for a period of several hours. The attack of asthma is not relieved as rapidly as when adrenalin is administered alone but the relief

is more prolonged. This mixture is only employed for two or three weeks because after this period the patient may begin to show loss of weight. Now adrenalin alone can usually be effectively administered in small doses. If it is found necessary to give more than one dose of adrenalin at fifteen minute intervals, then the possibility of the patient's acquiring adrenalin tolerance is very remote.

Concerning the after effects from the chronic use of adrenalin, I wish to state that more damage is inflicted upon the patient from the results of asthma rather than from the intelligent use of adrenalin. As a matter of fact the systolic blood pressure during an attack of asthma especially in patients past fifty years of age, frequently is raised twenty to sixty millimeters of mercury. In these patients adrenalin causes a marked reduction in blood pressure.

I cannot agree with the view that the restriction of adrenalin might be good practice in the patients taking the drug for a number of years. It is much more harmful to permit such patients to suffer through attacks of asthma with the additional danger of requiring the incidentally secondary organic changes in the lungs, bronchial tubes, heart, blood vessels and kidneys, I believe it is safer and more humane to give a patient as much relief from suffering as possible.

In a recent publication Dr. Howard Lillenthal of New York stated that asthmatic breathing can be relieved following massage of a site that has been injected with adrenalin several hours previously. His experience is limited to patients suffering with pulmonary tuberculosis. This procedure was carried out on a series of children with uncomplicated asthma with negative results.

Dr. I. S. Kahn, San Antonio, Texas.—I rather agree with Dr. Peshkin that these cases gradually in time develop more or less of a tolerance for adrenalin, and that is the reason they raise their doses. We usually get the history, as he said, of the attacks relieved by four, five, six or seven minims of adrenalin, maybe once a day, finally going up to three times a day, and many of these chronic adrenalin users, taking it for seven or eight years, have worked up to ten, twelve or fifteen minims, six or seven or even more times a day. As these cases of asthma are brought under proper allergy treatment, the doses of adrenalin are reduced.

With regard to Dr. Duke's suggestion of a heavy dose of aspirin and whisky, any of us would be glad to learn of any stunt that would help us out in cutting down adrenalin in severe asthma paroxysms. I have never tried a heavy dose of aspirin because I have always been afraid that a certain number of these cases might be sensitive to aspirin or other drugs, and have been afraid to try anything of the kind.

The thing that has given me most help in these cases has been small doses of ephedrin three times a day. The big trouble with these sick, chronic adrenalin using patients, is that they not only give negative skin tests as far as diagnosis is concerned, but such skin tests, even when negative, often appreciably increase the asthma. It has been my custom to try to get a diagnosis from the history and environmental study.

Regarding morphine, Dr. Duke told me a year or two ago that he never uses it, and Dr. Bulvert says he does not have to use morphine. If you go to a house and give fifteen or twenty minims of adrenalin for five or six doses, every twenty minutes or half hour, and your case shows no improvement, what can you do but give morphine? I do not see how we can get away from the use of morphine occasionally in a severe paroxysm of asthma. Of course, it is a bad thing to start. It is also true that a good many of these sick asthma patients will ask you not to give them morphine because they have an intolerance to it, it makes them sick, starts them vomiting, and they will beg you to hold off morphine just as long as you possibly can. I do not see, if you are going to handle very many sick asthmatic patients, how you are going to get away from the use of an occasional dose of morphine. In an acute attack of asthma, if adrenalin does not give relief after one or two repetitions, you are not going to get relief from adrenalin.

I had the pleasure of being in Dr. Duke's office a couple of years ago. He gave a beautiful demonstration of these physical agent cases of hay fever and asthma. He was able to produce hay fever of asthma by putting a patient under a lamp which gave out

heat or by rubbing the chest with ice. There isn't any question that he can bring on an attack of asthma and control it with heat and ice. I have always felt that he was dealing there not with the original basis of asthma but that his heat and cold sensitiveness was the same sort of secondary manifestation or secondary irritation that we could get in the cases which are brought on by paint, or varnish or spraying the nose with oil of wintergreen or camphor. I have always felt his conditions were secondary.

Dr Orville H Brown Phoenix Ariz—Just a word about adrenalin. I think in the main patients have a tendency to use too large doses of adrenalin when a small dose would be just as effective as a large dose.

Burkhart found that a half minim was just as effective as four or five or more. I make it a point to keep the dosage below that which will give any signs of an overdose causing trembling and so forth. I do not hesitate to repeat it as often as it is indicated in the smaller doses. I try to discourage going up over six or eight minims. Sometimes they seem to have an intolerance which makes it necessary to go above that but as a rule those who think they have to have those larger doses just think so and the smaller doses will be just as effective. I don't know how to explain it.

I have used pituitrin with adrenalin. I have a preparation put out by some firm in England. I had to pay a heavy duty on it for a while which made it very expensive but it certainly worked better than anything I ever used. It was said to be a combination of pituitary and ephedrin. In several severe cases which I had at that time it worked beautifully.

One thing that has not been mentioned here which I do wish to mention is the psychic state particularly in these cases which have not had asthma long. The psychic state plays a tremendous part. The cough is partly psychic and partly due to irritation from the mucus and everything that is in the bronchi. The cough will help to aggravate the existing condition. For instance you have a patient who is not in an attack but is wheezing and will say his asthma is sticking around. If you have him put on several forceful exhalations a half dozen you will have him in an attack of asthma.

I have had some results by using sodium iodide. Whether it is any better by vein than by mouth I do not know.

With regard to the use of aspirin I have not hesitated to use it in those cases who know they can use it. I have had about six or eight cases which were sensitive to aspirin and would have a tremendous edema or asthma or some disturbance from the use of aspirin, but in other cases one aspirin tablet a night, five grains would cause the patient to get along very comfortably.

With regard to Dr Duke's splendid work I am a little bit like the last speaker. There are a great many sensitive individuals that do have some sort of a change when a cold draft blows upon them from the window or from a fan. I do not know whether that is due to something that is separate and distinct from the process which we may call sensitization or not. I follow Dr Duke's work with a great deal of interest and I am afraid I am overlooking some of these cases. I was in his office last year and he told me he knew I was and I am certainly glad to have heard him this morning.

Dr Harry L Huber Chicago Ill—I should like to have Dr Duke answer one question. In these cases where he is using adrenalin, has he noticed a difference in its effect on patients with a high or low blood pressure? A large percentage of these asthmatics have very low blood pressures.

Dr Alexander Sterling Philadelphia Pa—I was under the impression that simultaneous administration of adrenalin and morphine is dangerous. I want to know whether or not it is so and whether Dr Duke had any serious results from the combined use of adrenalin and morphine.

The second point I should like to know is in the administration of aspirin in asthmatics is it in the sensitive or in the nonsensitive type that he has found it of benefit. I have had occasions to use aspirin in patients who have the inflammatory or infectious type of asthma.

whose asthmatic attacks alternate with mild or severe form of arthritis. Many of them do very well on aspirin in combination with some coal tar products.

Dr. William W. Dulc, Kansas City, Mo.—I hope I have not made myself misunderstood in giving this paper on the continuous use of adrenalin. I do not wish to be understood as saying that continuous use of adrenalin in small doses is harmful. I think the use of enormous doses is harmful, and I do think it is harmful in patients using 300 drops a day. I have had three patients who used from three to four ounces a day. Two of these patients using it in enormous doses were emaciated to skin and bones and they were just as brown as if they had Addison's disease. That is harmful and I believe it is useless.

There isn't a patient under my care who takes over one cc of adrenalin a day. I am always able to get by with a small dose.

Concerning adrenalin intolerance, it is perfectly remarkable that you can get these people taking three or four ounces a day down in a relatively short time to where one cc will do them as much good as the large dose previously.

What are we going to do with morphine? Try more whisky and aspirin, because it is actually more effective than morphine. I can tell you frankly that if you give aspirin and a good big dose of whisky, sometimes an ounce or even two ounces (and I think whisky is more easily taken if sugar is added and you can get along with a smaller dose if sugar is added, and you can also warm it up a bit which causes quick absorption, and we want a quick anesthesia), it actually is more helpful in my cases than morphine. I have a worse time with asthmatics addicted to morphine. I practically do not use morphine in my cases, and have not for a number of years. I do not think it is necessary.

Now, about the blood pressure. Remember, the majority of blood pressures are down. Adrenalin lowers blood pressure in asthma. I do not know what the relationship is between adrenalin and the blood pressure. I possibly was misunderstood if I led any one to believe that insulin, thyroid, peptone, or anything else takes the place of adrenalin.

With regard to stabilizing temperatures, I had one case which was subject to about fifteen or twenty short attacks of hives a day. If given a small dose of insulin in the morning, he would be free of his attack for four hours.

I had another case who was running a temperature and I believed she could be helped by changing her environment. She was living with a husband who was half insane and tormenting the dickens out of her. She was in a temperamental state of mind and everything would give her hives. When we got her away from home, however, her temperature did not sink to a low level.

With regard to pollen causing surface reactions, due to pollen sensitiveness, I have seen cases where they have changed and gone anywhere in the United States and still have asthma.

If you can make a person live a live on exposure to light and nothing else in the world, that person is sensitive to light. If you can bring out a hive by a drop of cold water on the skin, that is a cold sensitive case, and if you can cure these patients by eventually increasing tolerance to light and by gradually increasing tolerance to cold, you have as much reason for saying that is a light sensitive case or a cold sensitive case as you have for saying a pollen case that is relieved by pollen or by getting away from pollen, is a pollen sensitive case. There are so many types of heat and cold sensitiveness it makes it difficult. You can always relieve by cold water a pure cold sensitive case. It is perfectly remarkable what you can do with them.

Pollen is not absorbed from the skin by application of light to the skin, especially in the winter time when there is not a speck of pollen in the air.

Concerning the administration of morphine and adrenalin, I have never used morphine in such a case. I will say morphine is dangerous to give under any conditions. If you are not taking up heat and cold sensitiveness, I believe you are passing up something. I believe it is due to some change in the heat regulating mechanism. For instance, a person may have a severe case of scarlet fever and after that become sensitive to heat and cold. A cause of sensitiveness in one person will relieve it in another, for one person

can be in bed with the body warm and breathe cold air and have an attack. If you place a towel so they will breathe moist warm air the asthma goes. The next case will be precisely the reverse. They will have their attack if they are in a warm room breathing warm air and especially a little moist and have their body cold. The cure here is to open the window and let the party remain there.

I want to mention one thing which I forgot to put in my paper. It is one of the experiments I have been carrying out. In a closed room in one of our offices, the total number of pollen grains that fell on the half inch slide in that room during the entire year was thirty. Pollen settles to the floor just as dust, and if you keep the windows closed and let the pollen fall to the floor and have the room moist you will have a room practically free of pollen. If the air is in motion it stays up. If the air is absolutely still it stays down.

CLINICAL USE OF EPINEPHRIN IN ALLERGIC DISEASES

WITH SPECIAL REFERENCE TO A METHOD OF PROLONGING ITS EFFECT AND THE
IMPORTANCE OF ITS USE IN CASES OF ASTHMA COMPLICATED
WITH HYPERTENSION

By RAY M. BALSLEY, M.A., M.D., F.A.C.P., OKLAHOMA CITY, OKLA.

FOR the temporary relief of paroxysmal attacks of asthma no drug or combination of drugs has ever been added to the physician's armamentarium that equals epinephrin. For the purpose mentioned it is without question the drug par excellence. A newer drug, ephedrine, is a close second but is a much weaker dilator of the bronchial muscle. The action of epinephrin when given hypodermically is usually rapid and produces complete relaxation of the bronchial spasm but its effect is evanescent. The drug would be of much greater value in the palliative treatment of asthma if its effect were more lasting. Therefore, any practical method of prolonging its action would surely be news welcomed by both the patient and the physician.

Several years ago surgeons used epinephrin in connection with novocaine almost routinely as a means of constricting the blood vessels in the field of operation, thereby holding the local anesthetic longer. Many of them however have discontinued the use of epinephrin for this purpose because of the secondary hemorrhage which frequently appeared from six to fourteen hours following the operation.

Luckhardt and Koppanyi¹ have shown experimentally that epinephrin hydrochloride can be given hypodermically to dogs with a rise in blood pressure from 15 to 180 mm. of mercury a few seconds following the injection. The blood pressure would return to normal in from ten to twenty minutes. Several hours later when the area where epinephrin was given would be massaged the blood pressure would again rise in a few seconds but the extent of the rise would not be as great as the effect of the epinephrin given hypodermically.

Some weeks ago a nurse patient of mine, who suffers severely from asthma due to a Russian thistle sensitivity called attention to the fact that after taking epinephrin and obtaining complete temporary relief from the asthmatic spasm, she could obtain a somewhat similar relief from a subsequent spasm, if it appeared within six to ten hours, by thoroughly massaging the area where the epinephrin was given. In a recent clinical note Lihenthal,² a surgeon, called attention to some observations he had made on postoperative cases of tuberculosis who suffered from a bronchial spasm, and he reported that they obtained relief from the use of epinephrin, and on massaging the area where epinephrin was given several hours later, at the time a subsequent spasm appeared, relief from the same was obtained.

For several years we have used epinephrin with some of the higher doses of pollen extracts in certain cases for the purpose of constricting the blood vessels, thereby allowing the extract to go into the system very slowly. In this way we found we could prevent systemic reactions that would manifest themselves in the form of asthma, hives, or hay fever, that otherwise would appear. This led us to believe that epinephrin remained in the local tissue over several hours. The experimental work of Luckhardt and Koppanyi is excellent evidence that epinephrin remains in the local tissue for several hours and that it can actually be liberated into the blood stream by massaging the area where epinephrin was given. The experience of the nurse, which was mentioned, is clinical evidence that epinephrin remains in the tissues and can be forced into the circulation by massaging the area where it was given. Lihenthal's experience in his surgical cases who suffered from a bronchial spasm or from true asthma associated with tuberculosis, is clinical evidence that a certain amount of epinephrin remains locally in the tissue and it can be liberated into the blood stream by massaging the area where it was given.

The experimental and the clinical evidence mentioned encouraged us to investigate the value of the massage method of sending into the circulation epinephrin for relief of subsequent attacks of asthma. It occurred to us that if massaging the area several hours later where epinephrin had been given to the patient for relaxation of the bronchial spasm, would throw into the circulation a sufficient amount of epinephrin to relieve the subsequent attack when it appeared, it would be of great practical value not only to men dealing with asthma as a specialty but also to the general practitioner. This somewhat preliminary report that we are making is based on a study of a series of twenty cases suffering from paroxysmal attacks of true asthma.

METHODS OF STUDY

Patients who came to the Clinic suffering from attacks of asthma were first worked out from the standpoint of their sensitivity, and if the attack occurred during the day they were asked to come to the office, at which time eight minims of epinephrin was administered subcutaneously, with notations concerning the relief obtained, and the effect of the epinephrin on their blood pressure. They were asked to return, on the appearance of another attack so that the area could be massaged by us and the clinical effect on symptoms and blood pressure made. Those whose asthmatic attacks appeared at night

were advised to take eight minims of epinephrin, and on the occurrence of another attack to massage the area, instead of giving epinephrin again, and report to us.

The following case reports give some of the details of the results.

CASE 1—Mrs R L F, aged thirty five, had been suffering from moderate attacks of asthma each night for several weeks. She would frequently have to take a second dose of adrenalin. She was taught how to use her own adrenalin and was advised to follow the procedure as outlined above. Her attacks of asthma would occur about ten o'clock at night. She reported that adrenalin gave her almost instant relief but the attack recurred about three o'clock in the morning and on having the area massaged by her husband she obtained relief from the asthmatic spasm in three to five minutes. This procedure she repeated on a number of occasions, with relief each time. She found however on massaging the area twenty four hours later, or in other words ten o'clock the next night, she would obtain no appreciable relaxation from the bronchial spasm.

CASE 2—Miss M D, a nurse, aged forty, who had suffered from moderately severe attacks of asthma and had been using adrenalin for several months. She had learned several months ago that massaging the area where the adrenalin was given at the time the subsequent attack would appear, would give her relief if the second attack occurred the same night.

CASE 3—Mrs R K, aged thirty seven, has suffered severely from asthma due to Russian thistle sensitivity for the past three years. Her attacks would usually occur early in the night, and by the use of adrenalin she obtained relief. In the early hours of the morning, by following the instructions given her concerning the massage method she could obtain relief in from three to five minutes.

This patient was suffering from a severe attack of asthma while in the office for which eight minims of adrenalin was given. Before adrenalin was given her blood pressure was systolic 110, diastolic 80. Three minutes after the injection of adrenalin the blood pressure was 124 systolic, diastolic 80 and she complained of nervousness with a peculiar feeling in the abdomen of which a great many patients complain following the use of adrenalin. Four hours later while she was not suffering from asthma, the area where the adrenalin was given was massaged to find the blood pressure to change from 110/80 to 118/80. At this time she also observed the general nervous feeling and peculiar feeling in the abdomen, as she noticed at the time the adrenalin was given hypodermically.

CASE 4—Mr V C M, aged thirty eight, a patient we have observed for several months who recently has had to use adrenalin every night. He was given eight minims of adrenalin while suffering from an attack of asthma, by one of the members of the Clinic, on three occasions. Each time it was observed that his blood pressure would elevate from ten to twenty points above the normal from the use of the adrenalin. On massaging the area from four to eight hours later the blood pressure would elevate several points but not as much as at the time the adrenalin was given hypodermically. However at the time of massaging he would always have the nervous symptoms which he observed when adrenalin was first given.

His arm where adrenalin was given was massaged the second day that is twenty four hours later on two occasions, without any symptoms or without relief from the spasm for which he had come in complaining.

CASE 5—Mrs M R, aged fifty nine, an asthmatic patient of long standing who has been perfectly controlled so far as her asthma was concerned. To this patient adrenalin was given. Blood pressure readings were made and nervous symptoms observed. Her blood pressure changed from 140/80 to 144/80 following the use of adrenalin hypodermically. Eight hours later massaging the area where adrenalin was given changed the blood pressure systolic from 140 to 148 and produced marked nervous symptoms which she had also encountered at the time the adrenalin was first given.

CASE 6—R B, aged four, a doctor's son, whom I saw at ten o'clock one night, when he was given six minims of adrenalin, and I was immediately told by the mother that his attack would recur as they had been doing so every night, and therefore the boy would have to have another hypodermic of adrenalin. She was instructed to massage the arm thoroughly where the adrenalin was given and was told that it might relieve his attack, thereby not making it necessary to give the second hypodermic. The massage method was tried at five o'clock the next morning by the boy's father, a physician, with complete relief from the attack. The subsequent attack was very severe, awakening the boy from a sound sleep.

Other cases studied have given us somewhat similar results. In fact, but few cases on whom we tried the massage method or who used the massage method themselves within the first eight hours, failed to find partial or total relief from the asthmatic spasm.

From our findings it appears that massaging the area where epinephrin was given, from four to eight hours later, at the time the subsequent asthmatic attacks occur, will relieve the spasm in most cases. Those doing general practice who frequently see patients suffering from an acute severe attack of asthma, for example at ten to twelve o'clock at night, will usually find, if the attack is very severe, that it will recur from four to eight hours later. If such patients could obtain relief from the subsequent attacks by massaging the area thoroughly, it appears to the writer that it would eliminate the greatest objection to the use of epinephrin in the palliative treatment of asthma, namely, its evanescent action.

The series of cases we have studied up to the present, which is less than two dozen, is of course not sufficient to justify our drawing definite conclusions as to the value of prolonging the effect of epinephrin and thereby increasing materially its value in the treatment of asthma, but the results are so interesting and so full of possibilities that we feel justified in making this somewhat preliminary report.

USE OF EPINEPHRIN IN CASES OF ASTHMA COMPLICATED WITH HYPERTENSION

Since one action of epinephrin is that of elevating the blood pressure it is natural for one to feel that in case of asthma associated with hypertension it would be contraindicated. During the last eight years it has fallen to my lot to care for a number of cases of true asthma complicated with hypertension, also many cases of cardiac and cardiorenal asthma. For a long time we tried to stay away from epinephrin and use other means of controlling the asthmatic spasm in the cases complicated with hypertension. However, observing the wonderful relaxing effect of epinephrin upon the bronchial muscles in true asthma, we began to use routinely in very small doses this same drug in cases of true asthma complicated with hypertension and in the cardiorenal type of asthma, and we wish to report three cases which typify the results.

CASE 1—Dr A M, aged sixty-two, came to the Clinic suffering from seasonal hay fever and very severe paroxysmal attacks of asthma, of three months' duration. On examining him he was found to be extremely sensitive to pollen and also to animal dander. His blood pressure was 240 systolic and 100 diastolic. The urine examination was negative. The question naturally arose as to whether this man with a blood pressure of 240 should have adrenalin for the relief of his asthma. He was given five minims of adrenalin, and at the same time blood pressure observations were made.

During the twenty minutes following the injection of the adrenalin the blood pressure did not change. He obtained considerable relief from the asthmatic spasm. Two hours later eight minims of adrenalin was given with complete relief from the asthmatic spasm with an elevation of systolic pressure only four points. From that time on he was advised to use adrenalin himself as often as necessary for the relief of attacks, which he did and in three days his blood pressure readings were 180 systolic, 100 diastolic.

CASE 2—Mrs A. W. F. aged fifty-eight suffered with phthisis as a small child. During the last five years she has suffered from hypertension and moderately severe attacks of asthma. She was sensitive to animal epithelial only. Her physical examination revealed a blood pressure of 220/120. The liver edge could not be felt. The urine examination was normal. Here again the question arose as to whether adrenalin should be given for the relief of the asthmatic symptoms.

She was tested out with small doses of adrenalin hydrochloride and the blood pressure carefully watched without appreciable elevation. Larger doses were then used in sufficient quantity to relieve the attacks with elevation of the systolic pressure only from three to five points. By keeping down the attacks with the use of adrenalin her blood pressure finally stayed at 190/120. This patient of course was advised to rest a great deal and she was put on a low protein diet on general principles. During the last few months she has done exceptionally well for one with the complications she has had. She has to continue to use adrenalin from time to time for the relief of her asthmatic symptoms.

CASE 3—Mrs W. L. S. aged sixty-one came with a history of hay fever for twenty years and asthma for the past five years. Physical examination showed a blood pressure of 100/110 when he was not wheezing but during an attack he would have a blood pressure of 190/110. Here again adrenalin was used with relief of his attacks without dangerously elevating his blood pressure.

DISCUSSION

In cases of hypertension it is routine with most doctors to advise them not to get angry, not to walk against the wind, not to climb stairs, not to lift heavy loads, etc. In other words to do nothing that would have a tendency to elevate the blood pressure. We can conceive of nothing that would throw a greater load on the heart and would have a greater tendency to elevate blood pressure than severe paroxysmal attacks of asthma.

Our clinical observations on these cases have shown us that moderate size doses of epinephrin in cases of true asthma complicated with hypertension or in cardiorenal cases will raise the systolic blood pressure but little. Therefore, it seems to us that in cases of true asthma complicated with hypertension or in cases of hypertension with a failing heart or cardiorenal cases suffering from labored breathing paroxysmal in type, that some efficient means should be used to relieve the spasm. Knowing that there is no preparation that equals epinephrin for this purpose makes us feel that it should be tried out routinely in small doses and if it does not elevate the blood pressure appreciably it should be used for the relief of the asthmatic spasm. Assuming for the sake of argument that it does in some cases elevate the blood pressure, for example, even ten or twenty points, how much better it would be for epinephrin to elevate the systolic blood pressure which stands at 240 ten points for twenty minutes, but at the same time relieve them of their asthmatic spasm so that the pressure on its own accord would lower 50, 60 or even 80 points than it would be to allow the systolic blood pressure to stand at the height mentioned over a period of several hours while they are wheezing.

EPINEPHRIN IN HAY FEVER

Preparations of epinephrin, either aqueous or otherwise, in my opinion, should never, or seldom ever, be used on the mucous membrane of the nose of hay fever patients for the relief of congestion, since there is a secondary vasomotor dilatation which has a tendency to make symptoms more severe than they were before its use. It is fairly well recognized by the rhinologist that the continued use of epinephrin on the membrane of the nose does harm. For the temporary relief of eye symptoms in hay fever it should probably never be used.

During the efficient treatment of hay fever with potent pollen extracts it is not uncommon to encounter a systemic reaction, manifesting itself in the form of hives, asthma or hay fever, as one nears or reaches the tolerance dose, and for the relief of such symptoms there is no drug that will give better results than epinephrin given hypodermically in from ten to twelve minim doses. The untoward symptoms may reappear in from two to four hours, or even eight hours, and if they do, the area where the epinephrin was given may be massaged for relief, or a second dose of epinephrin can be given.

In pollen therapy epinephrin is also of considerable value in blocking the blood supply where some of the high doses of the extract are given, so as to allow it to enter the system more slowly, thereby preventing a systemic reaction. For example, certain patients may take 0.25 cc of the 1:500 dilution of an extract of grass or ragweed, or any other pollen, and from it encounter a systemic reaction. On dropping back a notch or two in schedule and building again they are not able to pass the same size dose without a similar experience. However, by drawing into the syringe, after the 0.25 cc of the pollen extract is in the syringe, three to four minims of epinephrin, and injecting that first into the subcutaneous tissue, and waiting a few seconds and then injecting the pollen extract, the systemic reaction will not be encountered, since epinephrin constricts the blood supply just as it does when the surgeon uses it along with novocaine, and thereby allows the material to enter the circulation over a period of several hours instead of most of it going in within a few minutes' time. On the next regular treatment day a dose of pollen extract still higher may be given, and a higher and still higher dose, etc., allowing one to carry the pollen extracts several doses higher in schedule, even up to a point where the patient will be fully protected from hay fever or asthma, who otherwise would not obtain relief from his hay fever or asthma symptoms on account of his tolerance dose being too low.

In at least 40 per cent of our hay fever cases we encounter systemic reactions before the patient is carried as high as we know they must be for protection. By means of epinephrin used as we have just outlined they can be carried sufficiently high to relieve them from their hay fever or asthmatic symptoms. It must not be forgotten, however, that when epinephrin is once used for the purpose of carrying the patient higher with pollen extracts, it must always be used.

EPINEPHRIN IN URTICARIA

For the temporary relief of acute attacks of urticaria due to food poisoning, serum sickness bites from insects etc., epinephrin in from six to twelve minim doses will usually give relief. However it may have to be repeated, and here again we have found that massaging the area where epinephrin was given, in the few cases we have tried will give relief from the subsequent symptoms that appear. If in cases of stings from insects or idiosyncrasies to foods or drugs there are symptoms of severe abdominal pain with swelling of the tongue or soft palate, epinephrin should be given in from ten to fifteen minim doses hypodermically and the throat should be swabbed with a 1:1,000 dilution. Several cases of death due to asphyxia have been reported from edema of the soft palate following stings from insects.

ASTHMATIC PATIENTS SHOULD BE TAUGHT HOW TO GIVE THEMSELVES EPINEPHRIN

It has been my custom during the last eight years to teach the asthmatic patients who come to our Clinic how to give epinephrin to themselves. Some doctors raise the question as to whether this should be done saying that patients might form the habit of using opiates and, too, that the danger of infection is great. During our eight years of care of allergic patients more than fifteen hundred cases of asthma have been studied and at least one third of these have been taught to give epinephrin to themselves. So far as we know not a single one of them today is a drug addict, and no case of infection has been reported. It is our opinion that the patient is going to use some drug for the relief of his symptoms, and therefore he should be taught how to use the best drug available.

For the relief of the asthmatic attack the best results are obtained by giving the epinephrin at the onset and not after the patient has wheezed for four or five hours. If they do not know how to give themselves epinephrin, they usually wait awhile to see if the attack will not subside and then a doctor is called, at which time the spasm is extremely severe. Frequently the doctor does not arrive for from two to six hours which means that the patient has suffered several hours from his asthma thereby not only depleting his system but producing emphysema. For the relief of such symptoms after the doctor arrives it will require from ten to twelve minims of epinephrin. The same relief in all probability could have been obtained with from four to five minims of the drug if given early. Many patients of course will suffer throughout the night instead of calling the doctor on account of financial circumstances.

Doctors so frequently tell patients that epinephrin will harden their arteries if taken over a long period of time and that it is a drug that is habit forming. Of course any drug that has to be taken frequently is probably somewhat harmful, some of them much more harmful than others of course, but since epinephrin is a normal constituent of the blood stream it will naturally have less deleterious effect on the patients than most drugs. Assuming that it might be somewhat harmful, how much less damage it would do than

the severe attacks of asthma that would continue for hours at a time. The end-results of unprotected asthma are emphysema, bronchiectasis and heart failure.

In teaching patients to give themselves epinephrin it has been our custom to have them always use alcohol for sterilizing the syringe and needle, as we feel that the danger of infection is much less than if the needle and syringe are boiled, as their fingers will have a less tendency to come in contact with the needle by this method of sterilization.

Asthmatic patients who have suffered severely over a period of time dread very much the thoughts of attacks. For this reason we teach our patients never to be without epinephrin. Even if they have been free several months they should carry the epinephrin and syringe for giving, and material for sterilization, when they go on their vacations or on business trips, so that they can always feel that they have at their command a product for the relief of the paroxysmal attack of asthma that might appear. Such an arrangement not only makes them feel more confident of themselves from the standpoint of doing the ordinary things of life that other people do, and thereby making men in business more practical, but they enjoy life so much more since they do not have to live in the dread of a severe attack of asthma without means of protection.

In about 85 per cent of the cases we studied, from 60 to 100 per cent relief from symptoms can be given but such relief is not obtained at once. It usually requires, for treatment of many cases of asthma, several months, and during this time it is of great importance that every attack of asthma possible be kept down by some simple means, and there is no drug of greater importance, and less harmful for this purpose, than is epinephrin.

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THE INFLUENCE OF ANIONS AND KATIONS ON THE VIABILITY OF *BACILLUS COLI**

By C H BOISSEvain, M D, AND ERIC WEBB, M D COLORADO SPRINGS COLO

FICKER¹ first showed the bacterioidal action of distilled water and physiologic salt solution. The viability of the cholera vibrio disappears after two or three days, if not more than 60,000 organisms are present in each cubic centimeter. In the presence of greater numbers a large percentage of them die in the first hours but the survivors multiply at their expense. The addition of 1 per cent nutrient broth to the 0.7 per cent NaCl solution also neutralizes the bacterioidal effect. Legroux and Ehava² found that the number of vibrio cholera, staphylococci and bacillus anthracis remains constant when suspended in distilled water to which 0.6 per cent of blood corpuscle extract had been added; the organisms die more or less rapidly if less extract is used. Panisset, Veige and Carneiro³ found that 0.8 per cent NaCl solution was more toxic than distilled water for staphylococci, typhoid coli, Friedlaender and anthrax bacilli. Loeb⁴ showed that the toxic effect of NaCl on living cells could be neutralized by the addition of appropriate concentrations of potassium or calcium salts. This suggested the work of Sherman and Holm^{5, 6, 7} and of Winslow and Falk⁸. They found that the toxic effects of NaCl may be neutralized by the addition of CaCl₂. The optimal concentration according to Winslow and Falk, is four parts NaCl to one part CaCl₂ with a total concentration of 0.725 M. The effect is most noticeable in alkaline solutions. Hotchkiss⁹ found that all chlorides prevent the growth of *B. coli*, if added in sufficient concentration to 1 per cent peptone water; the chlorides of the alkalis are least toxic, followed by the chlorides of the alkaline earths, while those of the heavy metals are most toxic. Shughnessy and Criswell¹⁰ found that the *B. coli* of their strain increased in number when suspended in distilled water, their distilled water was however, buffered by the addition of Northrop and DeKruif's buffer solutions which obviously introduces an error.

Most of the work on the effects of electrolytes on the viability of bacteria has been concerned with differentiating between the action of the cations, and with efforts to find 'balanced' solutions, in which the number of bacteria remains constant. Sherman and Holm⁵ find that Cl⁻, I⁻, NO₃⁻, SO₄⁻, PO₄⁻, accelerate growth of *B. coli* in peptone water, all other anions retard it. They found little difference between Na⁺, K⁺ and NH₄⁺; Mg²⁺ was more toxic for *B. coli*. Little other work has been done in regard to the anions. Falk¹¹ has a comprehensive review of the action of ions in physiology; he does not even refer to them. This lack of attention has been caused partially by the in-

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interesting discoveries of the physiologists about the antagonism between Na, K, and Ca in the contraction of smooth muscles, and partially by the attention that has been given in later years to the phenomena connected with Donnan's membrane equilibria Northrop and DeKam¹² showed that the isoelectric point of the typhoid bacillus is situated at a hydrogen-ion concentration of P_H 3.0. It follows from Donnan's equation, that only kations can influence the osmotic and electric properties of bacteria suspended in solutions more alkaline than P_H 3.0. It does not follow, however, that only the kations can be of importance for the viability of the bacteria. In applying Donnan's equation to bacteria, it is tacitly assumed that these are equally permeable for all simple ions. The possibility exists that this is not the case, and that the bacterial membrane is only, or at least mainly, permeable for anions, as is e.g., the case for erythrocytes. It is the purpose of this paper to show that the effect of the anions on the viability of *B. coli* is far greater than that of the kations.

We have limited our studies to the anions Cl^- , $SO_4^{=}$, NO_3^- , and $HPO_4^{=}$ and to the kations K^+ , Na^+ , Ca^{++} , Mg^{++} , as they seem most important from a physiologic point of view. Other factors included in the present study were the influence of the osmotic pressure and of the P_H in the hope of determining which part of the salt effect is due only to the chemical nature of anions, and which part is due to other factors.

METHODS

A strain of *Bacillus coli communis* was chosen for this work, because of its small tendency to spontaneous agglutination. In using staphylococci as some workers did, a complication was introduced by the possibility of a disintegration of the clumps of cocci, which would simulate an increase, when colonies were counted by the plate method. A twenty-four-hour broth culture was used, which was filtered through Whatman filter paper No. 5 and diluted 125,000 times, 0.1 cc. of this dilution was added to 8 cc. of nutrient agar at 45° C., plates poured and the colonies counted after twenty-four hours in the incubator at 37° C. This whole process of preparing the dilutions and pouring the plates did not take more than five minutes.

This method was preferred to the use of a washed and filtered suspension of bacilli grown on solid medium because the washing and centrifuging takes considerable time, preliminary experiments showed that the death rate of the coli bacilli in salt solutions is highest in the first hour, as shown in Chart I. In some experiments as many as 60 per cent died in the first hour, while a few of the more resistant bacilli were still alive after twenty-four hours. The difference between the different salt solutions must be most evident immediately after mixing the bacteria with the solutions. This difference in resistance that is seen between the single bacilli, can sometimes be observed between broth tubes inoculated with the same strain of bacteria. These changes in the percentage of resistant individuals in a broth culture affect uniformly the resistance against all salt solutions and do not affect the results. The amount of culture medium carried over in the 1/125,000 dilution is much smaller than

the 0.6 per cent necessary for the growth of bacteria in NaCl solution, as we could verify in experiments performed for that purpose

Bacilli remain alive longer in the same salt solution when kept in the ice box at 4° C than in the incubator at 37° C. All our experiments were performed at room temperature (20° C)

The salt suspensions with bacteria were kept in the dark as much as possible, because the diffuse daylight at the altitude of Colorado Springs (6000 feet) has a decided bactericidal action

Another point of some importance is the use of pipettes of uniform diameter. A considerable number of bacteria is always absorbed on the walls of the pipette and this effect will vary with its diameter. When several pipettes are used for succeeding dilutions the error increases in geometrical progression and may become large

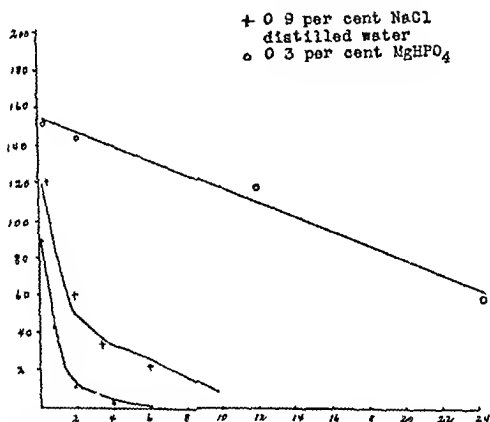


Chart I—Number of colonies grown from 0.1 cc. of 1/175,000 dilution in distilled water 0.9 per cent NaCl and 0.3 per cent MgHPO₄. The abscissa indicates the number of hours elapsed since making the dilutions and the ordinate the number of colonies

It seemed possible that the harmful effect of salt solutions was simply caused by the sudden transition from one medium to another. A bacillus that had been accustomed to a synthetic medium of a definite composition would in this case react differently to this salt concentration from a bacillus grown in broth. To determine this influence, we grew a colon bacillus through a series of seven transplants in a medium of the following composition

Asparagin	0.1 per cent
Glucose	0.1 per cent
MgHPO	0.1 per cent
KCl	0.1 per cent

Dilutions of these bacilli and of bacilli grown in ordinary broth were made in a medium of the following composition

MgHPO₄ 0.1 per cent
KCl 0.1 per cent

and the death rates in the two cases compared. The results are given in Table I. Since there is no appreciable difference between the death rate of the bacilli, we conclude that no error is introduced by using bacilli grown in nutrient broth.

TABLE I

NUMBER OF COLONIES GROWN FROM 0.1 c.c. OF DILUTION, WHEN THE BACILLI HAD BEEN GROWN IN DIFFERENT MEDIA

TIME ELAPSED SINCE MAKING DILUTIONS	BACILLI GROWN IN SYNTHETIC MEDIUM (DILUTION 1/12,500)	BACILLI GROWN IN BROTH (DILUTION 1/125,000)
0 hours	245	612
3½ hours	238	587
7 hours	150	522
24 hours	28	56

EXPERIMENTAL

Effect of osmotic pressure

A Nonelectrolytes—The effect of distilled water on the coli bacilli was compared with that of different concentrations of urea and glucose. The results are given in Table II.

The maximum number of survivors is in the urea solution of one-fourth molar concentration, both higher and lower concentrations are less favorable.

The results with glucose solutions are somewhat different. Less than one-fourth molar concentrations show the highest death rate, but no appreciable difference could be shown between one-fourth molar and higher concentrations. The most probable explanation seems that the glucose can penetrate into the bacilli and so equalize the difference in osmotic pressure, only the effect of the lower osmotic pressure can be demonstrated under these circumstances. It is interesting to note that neither of these solutions acts as a nutrient medium.

TABLE II

NUMBER OF COLONIES GROWN FROM 0.1 c.c. OF 1/125,000 DILUTION IN DIFFERENT UREA CONCENTRATIONS

TIME ELAPSED SINCE MAKING DILUTION	CONCENTRATION OF UREA				
	1/16 M	1/8 M	1/4 M	1/2 M	1/1 M
0 hours	211	207	182	204	185
2 hours	14	109	129	87	52
4 hours	2	8	19	5	1

NUMBER OF COLONIES GROWN FROM 0.1 c.c. OF 1/125,000 DILUTION IN DIFFERENT GLUCOSE CONCENTRATIONS

TIME ELAPSED SINCE MAKING DILUTION	DIST. WATER	CONCENTRATION OF GLUCOSE					
		1/50 M	1/16 M	1/8 M	1/4 M	1/2 M	1/1 M
0 hours	91	91	68	97	82	91	74
4 hours	1	1	1	7	15	27	14
24 hours	0	0	0	0	0	0	0

B Electrolytes—The effects of different concentrations of NaCl, KCl, CaCl₂, K₂SO₄, KH₂PO₄, NaH₂PO₄, were compared, in the case of the phos

TABLE III

NUMBER OF COLONIES GROWN FROM 0.1 cc OF 1/125 000 DILUTION IN SOLUTIONS CONTAINING DIFFERENT CONCENTRATIONS OF THE FOLLOWING SALTS NaCl KCl CaCl₂ K₂SO₄ MIXTURE OF Na₂HPO₄ AND NaH₂PO₄, MIXTURE OF KH₂PO₄ AND K₂HPO₄ (P_H OF ALL SOLUTIONS 7.0)

TIME ELAPSED SINCE MAKING DILUTIONS			CONCENTRATION OF NaCl			
DIST	WATER	1/50 M	1/16 M	1/8 M	1/4 M	1/2 M
0 hours	64	107	90	59	6	
8 hours	0	1	3	17	9	

TIME ELAPSED SINCE MAKING DILUTIONS			CONCENTRATION OF KCl			
DIST	WATER	1/50 M	1/16 M	1/8 M	1/4 M	1/2 M
0 hours	103	82	94	92	120	110
7 hours	2	1	3	51	34	7

TIME ELAPSED SINCE MAKING DILUTIONS			CONCENTRATION OF CaCl ₂			
DIST	WATER	1/50 M	1/16 M	1/8 M	1/4 M	1/2 M
0 hours	113	11	146	131	19	129
5 hours	0	0	26	9	1	0

TIME ELAPSED SINCE MAKING DILUTIONS			CONCENTRATION OF K ₂ SO ₄			
DIST	WATER	1/50 M	1/16 M	1/8 M	1/4 M	1/2 M
0 hours	160	255	273	202	11	21
5 hours	0	2	2	1	6	9

TIME ELAPSED SINCE MAKING DILUTIONS			CONCENTRATION OF Na HPO ₄ PLUS NaH ₂ PO ₄			
DIST	WATER	1/50 M	1/16 M	1/8 M	1/4 M	1/2 M
0 hours	87	132	208	122	324	104
8 hours	0	104	186	108	62	68
24 hours	1	69	541	431	299	90

TIME ELAPSED SINCE MAKING DILUTIONS			CONCENTRATION OF K HPO ₄ PLUS K ₂ HPO ₄			
DIST	WATER	1/50 M	1/16 M	1/8 M	1/4 M	1/2 M
0 hours	113	167	146	162	130	123
7 hours	0	8	181	228	207	97
24 hours	0	17	24	±3000	±1000	59

plates a mixture of primary and secondary phosphates of P_H 7 was used. The results are given in Table III.

The optimum concentration for most of these salts is at 1/8 M concentration. The only exception is CaCl₂ which has its optimum at 1/16 M. Theoretically we must expect an optimum at 1/12 M for all salts that dissociate in 3 ions. Only the calcium chloride gives evidence of a lower optimum than 1/8 M. The results are however complicated by the fact that the anions are responsible for the toxic action of the salts, as we shall show later. The toxic anion is approximately 2 times more concentrated in a calcium chloride solution than in an equimolecular sulphate solution, and its harmful effects must appear at a lower concentration.

EFFECT OF IONS IN EQUIOSMOTIC CONCENTRATION

A. Kations—The effects of 1/8 M solutions of NaCl and KCl were compared to 1/12 M solutions of CaCl₂ and MgCl₂. The results are given in Table IV.

Very little difference is found between the action of NaCl and KCl on one side and that of CaCl₂ and MgCl₂ on the other. The chlorides of the bivalent

salts are, however, more toxic for the colon bacillus than those of with univalent kations, the concentration of Cl^- ions being higher in equiosmotic solutions

B Anions—The effects of solutions of NaI , NaCl , Na_2SO_4 , NaNO_3 , and NaHPO_4 were compared, the results are given in Table V. The toxic effects of the anions depend apparently upon the chemical nature of the ion and no evidence is found of the valency effect that we found in the case of the kations. The phosphates are most favorable and even cause an increase in the number of bacilli. After the phosphates follow the ions NO_3^- , Cl^- , SO_4^- , I^- , in the order given.

TABLE IV

NUMBER OF COLONIES GROWN FROM 0.1 C.C. OF 1/125,000 DILUTIONS IN CHLORIDES WITH DIFFERENT KATIONS

TIME ELAPSED SINCE MAKING DILUTIONS	NaCl 1/8 M	KCl 1/8 M	CaCl_2 1/12 M	MgCl_2 1/12 M
0 hours	90	79	74	113
2 hours	71	59	3	13

INFLUENCE OF P_H

Sodium phosphate solutions of different P_H were compared. The results are given in Table VI.

The influence of the P_H of phosphate solutions is evidently of small importance as long as it remains above 5 and below 8.

ADDITIVE EFFECT OF IONS

In the first place physiologic salt solution was compared with Ringer's solution and with salt solutions to which only K or Ca has been added in the same concentration as in Ringer's solution. The results are given in Table VII.

There appears to be very little difference in the action of these solutions on the viability of the coli bacillus.

We next compared two solutions containing respectively 0.58 M NaCl and 0.145 M CaCl_2 , with a solution containing both. The results are given in Table VIII.

Winslow and Falk state that the solution containing both NaCl and CaCl_2 is less toxic than the solution containing only one of those salts. Our experiments give no evidence of "balancing" the toxic action of NaCl by that of CaCl_2 , the effects were purely additive.

Similar results were obtained by mixing equal parts of 1/8 M NaCl with 1/8 M NaNO_3 solution. The results are given in Table IX.

TABLE V

NUMBER OF COLONIES GROWN FROM 0.1 C.C. OF 1/125,000 DILUTIONS IN 1/8 M SODIUM SALTS WITH DIFFERENT ANIONS (IN THE CASE OF SODIUM PHOSPHATE A MIXTURE OF PRIMARY AND SECONDARY PHOSPHATES OF P_H 7.0 WAS USED)

TIME ELAPSED SINCE MAKING DILUTIONS	NaCl	NaI	NaNO_3	Na_2SO_4	SOD. PHOS.
0 hours	123	60	114	78	112
2 hours	82	0	105	52	116
5 hours	31	0	100	1	108

TABLE VI

NUMBER OF COLONIES GROWN FROM 0.1 c.c. OF 1/125,000 DILUTION IN 1/8 M SODIUM PHOSPHATE MIXTURES OF DIFFERENT P_H

TIME ELAPSED SINCE MAKING DILUTIONS	P_H OF THE SOLUTIONS				
	5	6	7	8	9
0 hours	76	108	83	106	103
4 hours	92	97	100	77	1
8 hours	10	136	194	13	0
24 hours	150	1200	5000	82	0

TABLE VII

NUMBER OF COLONIES GROWN FROM 0.1 c.c. OF 1/12,000 DILUTION IN 0.8 PER CENT NaCl SOLUTION AND IN RINGER'S SOLUTION

TIME ELAPSED SINCE MAKING DILUTIONS	0.8% NaCl	0.8% NaCl 0.0% KCl	0.8% NaCl 0.0% KCl 0.02% CaCl	0.8% NaCl 0.0% KCl	RINGER'S SOLUTION
0 hours	170	157	162	191	187
4 hours	68	73	27	6	56

TABLE VIII

NUMBER OF COLONIES GROWN FROM 0.1 c.c. OF 1/125,000 DILUTION IN 0.58 M NaCl 0.145 M CaCl AND 0.58 M NaCl PLUS 0.145 M CaCl

TIME ELAPSED SINCE MAKING DILUTIONS	0.58 M NaCl	0.145 M NaCl	0.58 M NaCl PLUS
			0.145 M CaCl
0 hours	107	99	97
4 hours	81	44	1
8 hours	38	2	1
12 hours	—	0	0

This experiment is not quite comparable to that of Table VIII. In that case we had, following the example of Winslow and Falk, added the CaCl to the solution containing the NaCl, thus changing the osmotic pressure. In the experiment of Table IX the osmotic pressure remained constant although half of the Cl ions had been replaced by NO_3^- ions. The mixture of chloride and nitrate was a little more toxic than the nitrate solution and a little less than the chloride solution.

If a sodium chloride solution is mixed with an equal quantity of sodium phosphate (P_H 7) the toxic effect of the sodium chloride is completely compensated as is shown in Table X.

The bacteria are even able to increase their number in the solution containing both phosphate and chloride. The increase was somewhat less in the solution containing both anions than in that containing only phosphates. The total increase may be compared with that shown in a 1/16 M sodium phosphate solution. Similar results were obtained when potassium phosphate was used or when sodium sulphate took the place of the sodium chloride.

INCREASE OF NUMBER OF BACILLI IN PHOSPHATE SOLUTIONS

The number of bacilli increased in several instances when they were suspended in phosphate solutions in the apparent absence of all nutrient constituents. We first thought that this might be due to an increase at the expense of dead bacilli and made serial transplants into other phosphate solutions. The increase could still be observed after the seventh transplant. We

now repeated the experiment in quartz tubes stoppered with glass wool. The number of bacteria remained now constant, and decreased proportional to

TABLE IX

NUMBER OF COLONIES GROWN FROM 0.1 CC OF 1/125,000 DILUTION IN 1/8 M NaCl , 1/8 M NaNO_3 , AND 1/8 M NaCl PLUS 1/8 M NaNO_3

TIME ELAPSED SINCE MAKING DILUTIONS	1/8 M NaCl	1/8 M NaNO_3	1/8 M NaCl PLUS 1/8 M NaNO_3
0 hours	97	61	75
2 hours	4	48	15
6 hours	0	7	1

TABLE X

NUMBER OF COLONIES GROWN FROM 0.1 CC OF 1/125,000 DILUTION IN 1/8 M NaCl SOLUTION, IN 1/8 M PHOSPHATE SOLUTION (MIXTURE CONTAINING PRIMARY AND SECONDARY SODIUM PHOSPHATE OF P_H 7.0), AND IN A 1/8 M SOLUTION CONTAINING EQUAL PARTS OF 1/8 M NaCl AND 1/8 M PHOSPHATE SOLUTION

TIME ELAPSED SINCE MAKING SOLUTION	NaCl 1/8 M	SOD PHOS 1/8 M	NaCl 1/8 M PLUS SOD PHOS
0 hours	76	55	55
6 hours	9	70	66
12 hours	0	90	73

the dilution in serial transplants. The colon bacillus is apparently able to satisfy its needs of nitrogen from the air, while bits of charred cotton fallen into the solution from the usual cotton stoppers are sufficient source of carbohydrates. This is especially remarkable when we remember that no increase in the number of bacteria took place in the urea or glucose solution. The only mineral constituent that the colon bacillus needs for reproduction is the phosphate ion. If sufficient phosphate is present, minimal impurities are sufficient for the life of the colon bacillus. The nature of the cation combined with the phosphate ion is without importance. We do not want to suggest that this is a general property of all bacteria, one of us¹³ has shown that the tubercle bacillus needs the presence of either potassium or rubidium for growth. Further study will have to show whether all bacteria need only the presence of phosphate ion to retain their viability or whether in some cases the presence of other ions may be necessary.

DISCUSSION

The influence of salt solutions on the viability is entirely determined by the nature of the anions. The addition of phosphate to the solution containing any of the other ions that we studied is sufficient to counteract its deleterious effect. This cannot be compared with the balancing of the Na ions by Ca or K in the fluid used for the perfusion of the surviving heart, in this case both ions are toxic, but their combination is not. In the case of the colon bacilli the phosphate solution does not possess any deleterious effect. On the contrary, we have shown that the presence of phosphate ions is necessary and sufficient for the survival of the colon

bacilli Part of the harmful effect of the other ions may be due to the fact that they permit the diffusion of the phosphate ions out of the bacteria, this is further confirmed by the fact that the addition of phosphate counter acts to a large extent the action of the other anions The action of the anions in solutions without phosphate is not entirely due to the absence of the phosphate ion, as is shown by the existence of the series of increasing toxicity NO_3 , Cl^- , SO_4 , I^- To explain the existence of this series and the action of the phosphates we must however, assume that the bacilli are permeable for anions No such assumption is necessary in the case of the cations The only difference observed was one connected with the valency of the cations A solution of CaCl_2 of the same molecular concentration as a NaCl solution contains twice as many Cl^- ions and must be more toxic If we decrease the molecular concentration we decrease at the same time the osmotic pressure below the optimum and the results must still be more unfavorable than in the case of the univalent ions

SUMMARY AND CONCLUSIONS

1 The optimum concentration for the viability of the colon bacillus is $1/4$ M in the case of nonelectrolytes and $1/8$ M in the case of binary electrolytes

2 The hydrogen ion concentration in phosphate solutions is of small importance as long as it remains above P_H 5 and below P_H 8

3 The cations are without effect on the viability of the colon bacillus, except for a valency effect

4 The series of increasing toxicity for the anions is HPO_4 , NO_3 , Cl^- , SO_4 , I^- Their effect is purely additive no evidence was found of 'balancing' the toxicity of one ion by that of another

5 The HPO_4 ion is the only mineral constituent necessary for the life of the colon bacillus Part of the toxicity of distilled water and salt solutions may be due to the fact that they permit the diffusion of the HPO_4 ion out of the bacteria

6 The number of colon bacilli remains constant in a solution containing a mixture of primary and secondary sodium phosphate of P_H 7 The number of bacilli increases if traces of organic material are present in the solution e.g., cotton fibers or dead bacilli

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THE RELATIVE TOXICITY OF GENTIAN VIOLET FOR CERTAIN MEMBERS OF THE COLON GROUP OF ORGANISMS*

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THE selective action of gentian violet for organisms having a gram-positive reaction was reported by Churchman, 1912. He studied the bacteriostatic effect of this dye and its relation to the gram reaction of the organism when the dye was added to the media in a dilution of one to seventy-five thousand.

Following Churchman's suggestion, Hall and Ellefson, 1917, report the use of gentian violet for isolating *B. coli* from milk. They added the dye to the broth to make a dilution of one to twenty thousand and reported a satisfactory result. They also reported, in 1918 and again in 1919, the use of the same dye in eliminating spurious presumptive tests for *B. coli* in water analysis. They suggested its use in routine bacteriologic examination of water and said, 1919, "One to twenty thousand gentian violet not only increases the total number of samples from which *B. coli* can be isolated, but reduces the number of spurious presumptive tests to a minimum." Winslow and Dolloff, 1922, reported the inhibitive concentration of gentian violet for *B. coli* in lactose broth as one part of the dye in fifty thousand parts of the media.

As a result of these reports we began, in 1924, to use gentian violet in the lactose broth used in the routine testing of water samples for *B. coli*. We concluded from the literature sighted that the best dilution of dye would be around one to forty thousand. We found that the dye when used in this manner exerted a marked inhibitory effect upon certain strains of *B. coli*. In 1918 we showed that of 200 cultures of *B. coli* isolated from as many samples of water, 60 per cent gave a positive Methyl red and negative Voges-Proskauer test. In as much as gentian violet has been recommended for its favorable selective action in isolation of *B. coli* and inasmuch as our results indicated that the dilution recommended undoubtedly inhibits certain strains, we decided to determine the relative toxicity of gentian violet on cultures of *B. coli* giving a Methyl red and Voges-Proskauer test.

EXPERIMENTAL WORK

In this work we subjected the cultures described above to various dilutions of gentian violet in lactose bouillon adjusted to P_H 7.0. The effect of the dye was determined by observing gas formation at the end of twenty-four and forty-eight hours' incubation, and by making again plates from the tubes at these intervals and counting the colonies growing at the end of

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forty eight hours. The inoculations of the gentian violet lactose bouillon tubes were made as follows. A twenty four hour growth on plain agar slants was washed down with two cc of sterile salt solution. A loopfull of this suspension was transferred to ten cc of sterile salt and shaken well. From this suspension a tube of plain nutrient broth was inoculated with one loopfull and allowed to incubate twenty four hours. A loopfull of the twenty four hour plain nutrient broth culture was transferred to ten cc of sterile salt and shaken well. This suspension was used to inoculate the gentian violet lactose broth tubes, using one loopfull to each tube. At the same time a lactose broth fermentation tube without gentian violet was inoculated in a similar manner. The tubes were incubated for forty eight hours being observed for gas production after twenty four and forty eight hours' incubation. At the same time twenty four and forty eight hours, agar plates were made from each tube and counted after forty eight hours' incubation. The dilutions of gentian violet in the media ranged from a strength of one part of dye to one hundred thousand parts of media to one part of dye to ten thousand parts of media.

Agar plate counts were made on the suspensions used to inoculate the gentian violet tubes so that we might be sure that the fermentation tubes were receiving a large enough inoculation. While the results of these counts do not appear in the tables they ranged from five hundred and forty to four teen hundred colonies to the plate. It seems probable, then, that no fermentation tube in the experiment received an inoculation of less than five hundred and forty bacteria.

The result of the inoculation of the lactose bouillon fermentation tubes containing no gentian violet are not included in the tables. These are omitted because all of the tubes in which the gentian violet was diluted highly (1:100,000) showed gas production in twenty four hours and acted as a control for the rest of the experiment. However, all plain lactose broth tubes showed gas production in the first twenty four hours.

RESULTS

Table I shows the results of the agar plate counts. These results show that the dye when diluted one hundred thousand times produced no inhibition, which could be detected by the method used, of either group of organisms. That is there was always from these tubes a good growth on the agar plates. Sometimes the number of colonies which grew on the plates made from the gentian violet tubes were less than the number which grew on plates made from the plain lactose bouillon but these differences were small. Table I shows that in a dilution of one to seventy five thousand, one culture of the methyl red group failed to grow. At a dilution of one to sixty thousand three cultures failed to grow. At a dilution of one to thirty thousand, the dye showed a 90 per cent inhibitory effect while at a one to fifteen thousand no culture grew.

The result with the Voges Proskauer reacting organism shows no inhibitory effect until a dilution of one to thirty thousand is reached and then only one to the eight cultures used failed to grow as against a 90 per cent

TABLE I

TABLE SHOWING INHIBITORY EFFECT OF GENTIAN VIOLET LACTOSE BROTH AS INDICATED BY AGAR PLATE COUNIS MADE FROM BROTH AT END OF TWENTY FOUR AND FORTY-EIGHT HOURS

a Methyl Red Positive Organisms

CUL NO	1-100000		1-75000		1-60000		1-40000		1-30000		1-20000		1-15000	
	24	48	24	48	24	48	24	48	24	48	24	48	24	48
1	540	330	6	580	0	0	0	0	0	0	0	0	0	0
2	580	570	2	600	0	0	0	0	0	0	0	0	0	0
3	1050	360	2	1400	0	14	0	6	0	0	0	0	0	0
4	360	220	0	0	0	0	0	0	0	0	0	0	0	0
5	1050	440	480	1050	990	4200	380	380	0	0	0	0	0	0
6	2100	900	500	840	250	700	6	400	0	0	0	0	0	0
7	1400	1050	0	1750	1	1260	0	0	0	0	0	0	0	0
8	1400	540	15	680	2	1750	0	0	0	0	0	0	0	0
9	2100	750	460	0	880	840	140	350	160	730	0	280	0	0
10	480	280	480	0	640	840	0	0	0	0	0	0	0	0

b Voges Proskauer Organisms

11		770	1050	1050	840	360	700	90	360	21	210	1	18
12		840	1070	840	700	510	520	200	370	28	310	5	38
13		740	1400	540	490	300	420	0	0	0	0	0	0
14		940	2100	530	700	320	--	110	110	0	0	0	0
17		560	460	1050	440	600	380	0	0	0	0	0	0
18		1400	1260	1750	1400	1050	650	1400	1700	96	1260	19	700
19		540	1400	280	560	450	520	320	670	0	52	0	0
20		520	1500	480	490	630	1050	0	270	0	0	0	0

inhibition for the methyl red positive organisms. It is also seen from this table that at a dilution of one to fifteen thousand, the point at which every methyl red organism was prevented from growing, only a 62 per cent inhibition of the Voges Proskauer organisms was apparent.

A further study of Table I shows that in a one to one hundred thousand dilution of the dye no evidence of lag of the growth was apparent. In this dilution the number of bacteria growing in the tubes after twenty-four hours' incubation as indicated by the number of colonies on the agar plates is slightly greater than after the second twenty-four hours. This was also true of the plates made from the tubes containing no gentian violet. This would seem to indicate that the maximum bacterial growth was reached in these tubes in the first twenty-four hours. From this dilution (1-100,000) down, every dilution, except on cultures 9 and 10, shows an evidence of lag in the first twenty-four hours in the methyl red group. This evidence was more apparent as the amount of the dye in the media was increased.

The organisms of the Voges-Proskauer group do not show this lag until a relatively low dilution (1-30,000) is reached. The counts from the tubes containing the higher dilutions of dye are so nearly alike or are so high both after twenty-four- and forty-eight-hour incubation that no inference can be made by their comparison.

It is apparent that a few of the organisms in each group showed more resistance to the dye than other members of the same group. This is seen by comparing cultures No 1 and 2 with cultures No 5, 6, and 9 of the methyl red group. Culture No 1 began to show marked sensitiveness to the dye in dilution of one to seventy-five thousand, while the other cultures showed

a good growth on the plate until a dilution of one to forty thousand was reached. On the other hand, culture No 9 was particularly resistive, continuing to grow until a dilution of one to fifteen thousand was reached. The sensitiveness of cultures No 1 and 2 is also seen by a comparison of the *twenty four and forty eight hour counts from the tubes containing a one to seventy five thousand dilution of gentian violet*. This shows that after twenty four hours' incubation, the plates from these tubes grew only six colonies while from these tubes at the end of forty eight hours, the plates grew 580 colonies. This is interpreted to mean an inhibition by the dye causing a lag in the growth of the organisms for the first twenty four hours.

Table II shows the effect on gas production from lactose by cultures of *B. coli* when gentian violet is added to the lactose bouillon.

TABLE II

SHOWING THE EFFECT OF VARIOUS CONCENTRATIONS OF GENTIAN VIOLET ON CULTURES OF *B. COLI* AS EVIDENCED BY THE PRODUCTION OF GAS IN GENTIAN VIOLET LACTOSE BROTH

Twenty Four Hour Gas Production

CONCENTRATION GENTIAN VIOLET	POSITIVE METHYL RED ORGANISMS										NEGATIVE VOGES PROSKAUER ORGANISMS									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	18	19	20		
1 to 100 000	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-		
1 to 75,000	0	0	0	0	+	+	0	0	0	+	+	+	+	+	+	+	+	+		
1 to 60 000	0	0	0	0	0	0	0	0	0	0	+	+	0	+	+	+	0	0		
1 to 40 000	0	0	0	0	0	0	0	0	0	0	+	+	0	+	+	+	0	+		
1 to 30 000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0		
1 to 25 000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
1 to 20 000	-	-	-	-	-	-	-	-	-	-	0	0	-	0	0	0	0	0		
1 to 15,000	-	-	-	-	-	-	-	-	-	-	0	0	-	0	0	0	0	0		
1 to 10 000	-	-	-	-	-	-	-	-	-	-	0	0	-	0	-	0	0	0		

Forty Eight Hour Gas Production

1 to 100,000	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
1 to 75,000	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1 to 60,000	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1 to 40,000	0	0	0	0	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+
1 to 30,000	0	0	0	0	0	0	0	0	+	0	+	+	0	+	0	+	+	+	+
1 to 25,000	0	0	0	0	0	0	0	0	0	0	+	+	0	+	0	+	+	+	+
1 to 20,000	-	-	-	-	-	-	-	-	-	-	0	+	-	0	0	+	0	0	0
1 to 15,000	-	-	-	-	-	-	-	-	-	-	0	0	-	0	0	+	0	0	0
1 to 10,000	-	-	-	-	-	-	-	-	-	-	0	0	-	0	-	0	0	0	0

0 per cent or more was considered gas formation

These results were observed in the same tubes from which the agar plate counts were made and the results are recorded so that the cultures have the same number. A study of this table shows that all of the methyl red cultures produced gas in the first twenty-four hours in the tubes containing the dye in a dilution of one to one hundred thousand, and that all but 3 failed to produce gas in the first twenty-four hours when exposed to the effect of the dye in a dilution of one to seventy-five thousand. There was a complete absence of gas in all methyl red organisms in the first twenty-four hours' incubation in the presence of the dye in the proportion of one part of dye to sixty thousand parts of media. This table also shows what the bacterial counts showed, i.e., that in the case of the methyl red organisms the gentian violet produced a period of lag after which growth takes place faster. The methyl red positive organisms show this more than the Voges-Proskauer organisms. Evidence of this is seen in the table by an absence of twenty-four-hour gas production, and its production in forty-eight hours in many of the cultures. It is also apparent from this table that some cultures are more resistant to gentian violet than others. This is evidenced by cultures 5, 6, and 9 which continued to produce gas in forty-eight hours after the others had been completely inhibited. These are the same organisms which showed stronger resistance by the plate counts.

The organisms of the Voges-Proskauer positive group were not inhibited as evidenced by gas production until a dilution of one to sixty thousand was reached, when three cultures (13, 19, and 20) failed to produce gas in the first twenty-four hours. In this group there is no general lag effect, as evidenced by an absence of twenty-four-hour gas until the dilution of the dye had reached one to thirty thousand.

SUMMARY

Gentian violet has a marked inhibitory effect on the growth of *B. coli* in a dilution of one to twenty thousand. In this strength it prevented growth in 90 per cent of the methyl red and 50 per cent of Voges-Proskauer cultures used in our tests. The inhibitory effect was evident on the methyl red positive cultures in a dilution as high as one to seventy-five thousand, while it appeared not to affect the Voges-Proskauer positive group until a very much lower dilution (1:30,000) was reached. The inhibitory action is noticed in the higher dilutions by the lag produced in the growth of the cultures. This lag effect is seen in the methyl red positive in much higher dilutions than in the Voges-Proskauer positive cultures. There is a marked difference in the resistance of the two groups of organisms (methyl red and Voges-Proskauer) to the dye. Within the same groups there is a variation in sensitiveness of the the organisms to the action of the dye.

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HEMOLYTIC ICTERUS RESEMBLING PERNICIOUS ANEMIA*

By WM ALLAN M.D., CHARLOTTE, N C

HEMOLYTIC icterus at times produces remarkable blood pictures that make diagnosis difficult. We have previously reported a case¹ in which the blood picture following an almost fatal hemolytic crisis resembled a combination of myeloid leucemia and pernicious anemia. Krumbhaar² points out that the cases with severe anemia may so resemble pernicious anemia that differentiation is difficult as in the case here presented.

Case history—A white farmer aged 31 single was referred to me April 28 1924 by Dr A F Thompson of Troy N C. This young man looked about sixteen years old was tall, thin, and ghastly pale with lemon tinted skin and with deeply bronzed face and hands, he was very weak and short of breath and his ankles were considerably swollen. He gave a history of paleness and weakness for eighteen years following whooping cough at thirteen but later his father said he had been pale long before that. From the age of fourteen to nineteen he had a breakdown every summer for six years, and for the next twelve years he had a breakdown about every second year. The present breakdown in health dates back about four months during which time he has had every ten days to two weeks headaches and bilious attacks which seem to hold him back. There is no history of any jaundice or anemia in parents sibs or other members of the family and the father and several sibs that were seen from time to time showed no evidence of jaundice or anemia so far as inspection went nor did they have palpable spleens. The only history of weakness before the patient's chronic trouble started is that of several attacks of bloody flux. Physical examination showed dull muddy looking conjunctivae but no distinct jaundice although the skin had a noticeably yellowish tinge with deep brown pigmentation of hands and face. The throat and lungs were clear. Heart size and sounds were normal regular rate 90. Blood pressure 100/50. Abdomen full with thin walls spleen extended to the crest of the ilium with sharp edge smooth surface and not tender. The lower edge of the liver was just palpable. Reflexes normal with very poor musculature and nutrition. Blood examination showed hemoglobin 30 per cent red cells 1,352,000 giving color index of 1.1 leucocytes 6,300, with polynuclears 72 per cent, lymphocytes 25 per cent eosinophiles 1 per cent basophiles 1 per cent microcytes 1 per cent. The red cells showed very marked variation in size shape and staining reaction and a moderate number of normoblasts were seen. In testing the red cells for fragility hemolysis began at 0.46 per cent and was complete at 0.32 per cent salt solution. The blood serum was slightly positive for bile. The urine was strongly positive for urobilinogen, but otherwise negative. The patient seemed too weak and short of breath to justify analysis of the gastric juice.

This hemolytic anemia starting in early youth and running a course marked by remissions and exacerbations for two decades suggested pernicious anemia but as only about 1 per cent of pernicious anemia begins so early in life³ and as only about the same proportion⁴ has very large spleens this diagnosis did not seem probable. On the other hand the negative family history and the lack of increased red cell fragility made the diagnosis of hemolytic icterus too uncertain to advise splenectomy in a very poor surgical risk.

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Blood transfusion followed by iron and arsenic and increased diet for six weeks accomplished nothing as the blood picture on June 11 was practically the same as when first seen. It was evident that a continuation of symptomatic treatment was useless.

The only chance of removing the cause, or at least the locus, of the hemolysis here seemed to be by splenectomy, as the beneficial effects of this measure in both the familial and acquired types of hemolytic jaundice are well known. The lack of the pathognomonic increased fragility of the red blood cells in this case was considered explicable by the observations of Giffin and Sanford⁴ that "the increased fragility of hemolytic jaundice can be modified by long continued anemia even to the degree that an increase of resistance may be present."

After two transfusions this large spleen was rapidly removed by Dr. A. G. Brimzer, June 17, and the patient made a good surgical recovery. By the end of the month the hemoglobin had reached 65 per cent and the red cells were 4,336,000. In October, 1924, the patient showed a ruddy skin with a gain of twenty pounds in flesh and a corresponding gain in strength. Early in 1926, his hemoglobin was 84 per cent, red count 6,400,000, leucocytes 15,000, with polynuclears 66 per cent, lymphocytes 30 per cent, eosinophiles 3 per cent, basophiles 1 per cent.

A case of severe hemolytic jaundice of long duration strongly resembling pernicious anemia is reported in which the usual increased erythrocyte fragility was possibly counteracted by long-continued anemia.

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LABORATORY METHODS

USES OF THE BIOLOGIC COLORIMETER AS A STAND FOR THE HAND SPECTROSCOPE*

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IN THE quantitative estimation of certain substances methods have been employed which are based upon the extinction of spectral bands by dilution. The accuracy and delicacy of such determinations are not dependable due to the absence of a standard degree of illumination and the change of characteristics of the absorption spectrum upon dilution. It is preferable to use solutions of similar concentrations in any series of comparable analyses. The spectral intensity can be determined by varying the thickness of the layer of liquid which is interposed between the slit of the spectroscope and the source of illumination, using as an end point either the point of disappearance of the absorption band or by matching its intensity with that of a standard solution set at a suitable depth of liquid.

For several years one of us has been using a modified colorimeter for this purpose. The first model described in this journal¹ was soon superseded by an adaptation of the Duboscq colorimeter which had a greater range of applicability. The eyepiece of the Duboscq (Pellin) colorimeter was temporarily removed and a hand spectroscope inserted in its place. By exposing the prisms and sliding them to one side so that the dividing line just passes out of the field of vision and the light comes entirely through one cup, a single spectrum is obtained while by leaving the prisms in their regular position and adjusting the spectroscope so that the spectrum is vertical to the field of vision, two spectra will be seen lying side by side. Recently Bausch and Lomb have constructed two instruments for us which are an outgrowth of this earlier instrument employed by one of us (B).

In the first of these instruments (see Fig. 1) designed by Myers the eyepiece and prisms of a B. and L. biologic colorimeter were replaced by a hand spectroscope on a special mounting. The spectroscope has a fixed aperture and a metal eyeshield. The two plungers and cups of the colorimeter are replaced by a single plunger and cup which together with the rack and pinion are centered directly below the spectroscope. This is a particularly convenient mounting for a hand spectroscope since the concentration at which characteristic absorption bands occur may be almost instantaneously obtained by raising or lowering the cup. When a solution showing absorption bands

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for example oxyhemoglobin, is placed in the cup, the variations in these absorption bands may be quickly studied by raising and lowering the cup, also the disappearance point of the absorption bands may be readily determined. When the disappearance of absorption bands is made the basis of a quantitative method, as for example in the spectroscopic estimation of urobilin, this instrument is a very convenient one to use. With constant intensity of illumination and a similar initial dilution, successive results are comparable and quite accurate.

The second of these instruments (see Fig 2) was designed by Boyd as an accessory attachment for the standard colorimeter. By replacing the eyepiece of the latter with this adaptation, the instrument becomes a spectro-

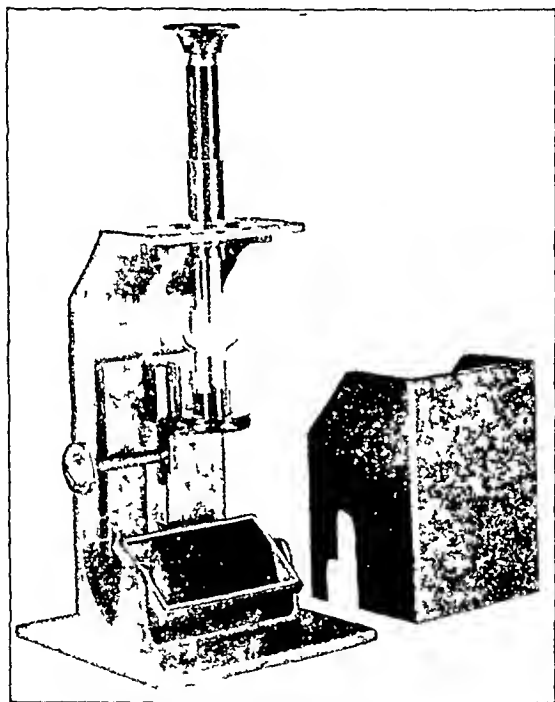


Fig 1—Hand spectroscope mounted on stand of B and L biologic colorimeter

comparator. The adapter consists of a hand spectroscope mounted in a tube which is of the same diameter as the eyepiece, it is equipped with an accessory lens which increases the spectral dispersion. The spectrum lies vertical to the plane of vision, the red field being uppermost. Light entering the instrument through the two glass plungers gives rise to two spectra lying side by side, as readily comparable as are the two fields of the colorimeter. The instrument may be used as the one described above, using the blank side as a control. Greater accuracy can be obtained, however, by using a standard solution of known concentration in one cup, set at a suitable depth, and matching the intensity of the bands of the unknown solution with this. As it is necessary to shield the eye from other sources of light while using the instrument, a special rubber eyeshield has been devised for this purpose.

In comparison with the instrument shown in Fig 1 the spectrocomparator has two important advantages, (1) that a special instrument is not required, since the conversion from a colorimeter to a spectrocomparator (Fig 2) or vice versa requires only a few seconds and (2) that a control spectrum or the absorption bands of a standard are always in the same field of vision with the unknown. It has the disadvantage that the spectrum field observed is considerably smaller. For purely qualitative work the instrument shown in Fig 1 is probably preferable.

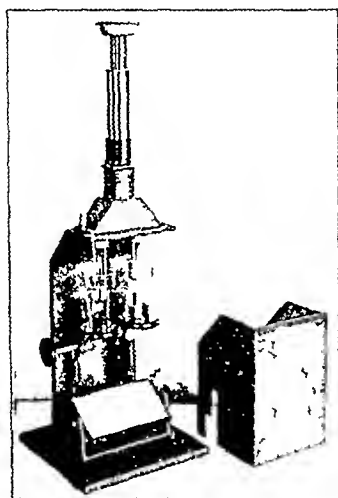


Fig —The spectrocomparator an adapter for the colorimeter

As a quantitative clinical method the spectroscopic method of measurement has been used chiefly in the estimation of urobilin by the Wilbur and Addis technique. In the estimation of hemoglobin it checks quite well with the colorimetric methods. It would seem quite applicable to the determination of any substance yielding definite absorption bands even in the presence of colored contaminants, provided that the absorption spectrum is not obscured.

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THIOCYANATE AS A SOURCE OF ERROR IN THE FERRIC CHLORIDE TEST FOR LACTIC ACID, WITH A METHOD FOR THE ELIMINATION OF THE THIOCYANATE*

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IN THE course of routine tests for lactic acid in human gastric juice, it was found that in many individuals a thiocyanate was present in the samples, and that this was a source of error since it gave a color with ferric chloride somewhat similar to that given by lactic acid. Since this must be a very common contamination, and since it is not mentioned in those texts on Clinical Diagnosis which were consulted, it appeared advisable to call attention to this source of error and to a method by which it may be avoided.

KCNS is commonly present in human saliva. Schneider¹ tested 225 cases and in only one did he find no KCNS. He found it to be present in greater concentration in the saliva of smokers than in that of nonsmokers. Kelling² reports that he found KCNS in the gastric secretion. In very small concentrations KCNS gives with ferric chloride a yellow color somewhat more brown than that given by lactic acid. In greater concentrations the KCNS gives a brown or brownish red which may entirely obscure any yellow lactic acid color. Thus it is a source of error both by simulating the lactic acid color and by obscuring it.

While KCNS is generally known to be present in saliva, it is not generally recognized as a constituent of gastric juice. Tests on the gastric contents of human subjects were carefully made to avoid any contamination of the gastric secretion by saliva (the saliva was expectorated and none was consciously swallowed) and still KCNS appeared in the gastric contents. No quantitative tests were made, but the concentration in gastric juice seemed to be less than in the saliva of the same individual. In order to be sure that the gastric secretion was not being contaminated by KCNS bearing fluids from the mouth or duodenum, a Pavlov pouch dog was injected subcutaneously with 0.1 gram of NaCNS after the gastric glands had been stimulated by a meal of meat, and the KCNS content of the gastric juice was followed at half hour intervals. One-half hour after the injection of the NaCNS the gastric juice gave a very faint test for KCNS, one hour after the injection the test was very strong, and one and one-half hours after the injection it was still quite marked.

It was found that the KCNS could be precipitated by the heavy metals, and that the best substance for this purpose is HgCl_2 , in saturated solution. I later found that Kelling² had previously used the same method for the elimination of KCNS.

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Elimination of KCNS by HgCl_2 solution is applicable to any of the common tests for lactic acid. A saturated solution of the mercury salt is used. If saturated HgCl_2 be added to a solution of KCNS no color is present, and on the further addition of ferric chloride the solution takes on only the very faint brown caused by the ferric chloride itself. If lactic acid and KCNS are present in the same solution there is no change in color on the addition of a saturated solution of HgCl_2 , but on the addition of ferric chloride the typical lactic acid color develops. If Uffelmann's test is used the gastric content is treated with an equal amount of saturated HgCl_2 before adding it to the Uffelmann's reagent. If Strauss' test (extraction with ether) be used, the elimination of thiocyanate is equally as important as with the more simple tests, since the thiocyanates are very soluble in ether. If an amount of saturated HgCl_2 solution equal to that of the solution to be tested is added prior to the ether extraction, the extract will be free from KCNS.

The addition of HgCl_2 in no way decreases the sensitiveness of the test for lactic acid except as it dilutes the material to be tested. If the material to be tested is neutral or alkaline it should be made acid with HCl before the addition of the HgCl_2 , this serves the double purpose of liberating lactic acid from the proteins and avoiding precipitation of mercury by alkali.

It is hoped that this method of avoiding a common source of error, especially in patients who smoke will increase the value of the test for lactic acid. The organic acids and the fatty acids still remain as contaminations. However they are not usually found in the stomach following the test meal unless fermentation is taking place.

SUMMARY

It is pointed out that KCNS is a common and generally unrecognized contamination in the ferric chloride test for the presence of lactic acid in gastric contents and may lead to incorrect conclusions especially in patients who smoke. It is shown that KCNS is secreted by the gastric glands. The addition of saturated HgCl_2 solution prior to the performance of any of the common tests for lactic acid will remove the KCNS.

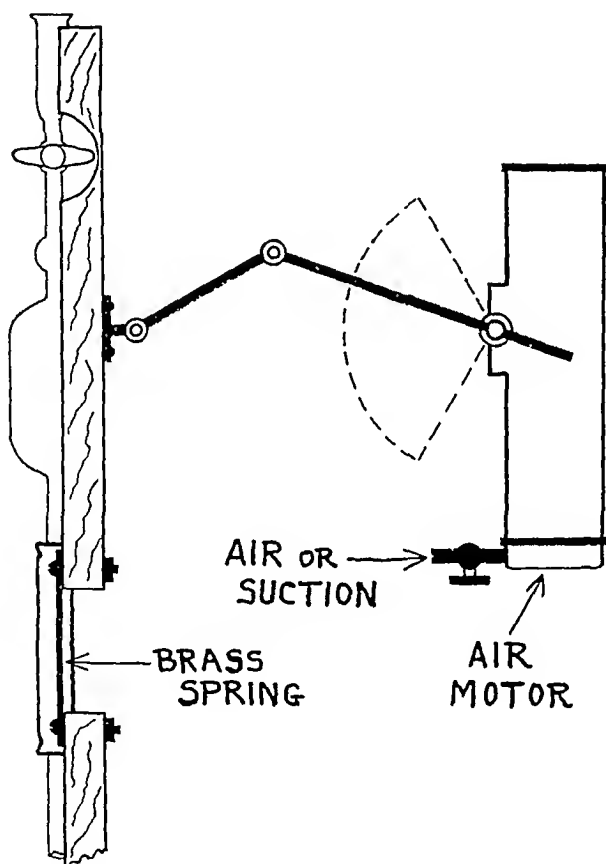
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AN INEXPENSIVE SHAKER FOR THE VAN SLYKE BLOOD GAS APPARATUS*

BY H. F. PIERCE, BALTIMORE, MD

SINCE the Van Slyke blood-gas apparatus, of the constant volume type is now almost universally employed in hospitals and other medical institutions, the simple and inexpensive shaking device here illustrated may be of interest to technicians and other users of the apparatus



As is shown in the diagram, the gas bulb is mounted on a board, which is attached to the frame supporting the rest of the gas apparatus by two narrow strips of thin spring brass, one on either side of the rubber connecting tube. To the back of the board carrying the gas bulb is attached a jointed connecting rod, the far end of which is fastened to the shaft of a reciprocating air motor such as is commonly used to operate automobile windshield wipers.

*From the Wilmer Ophthalmic Institute, The Johns Hopkins Hospital.
Received for publication, November 21, 1927.

The connecting rod should be mounted so as to swing through the arc indicated by the dotted lines in order to obtain the maximum shaking effect. Power for the air motor is obtained from the suction of a large water aspirator of the usual kind. A similar motor designed to operate on compressed air is on the market, and may be used if the laboratory is provided with air mains. In choosing a suitable motor, care should be taken to select one powerful enough to do the work.*

Apparatus fitted with this device has been in use for over a year and has yielded results which check with those secured from a similar apparatus provided with an electric motor shaker.

A SIMPLE AND EFFICIENT APPARATUS FOR THE DISTILLATION OF UREA NITROGEN†

By LYMAN C. MURPHY AND ROBERT C. JENKINS, CHICAGO, ILL.

WHILE it must be acknowledged that the distillation method of Folin and Wu for the determination of urea nitrogen is more accurate and expeditious than aspiration, many technicians have discarded the former method for the latter owing to the difficulty of preventing the liquid in the receiving tube from sucking back, or to the tendency of the solutions in the distilling tube to froth over.

We have found that by constricting the delivery end of the connecting tube, thereby maintaining a gentle but fairly uniform pressure within the apparatus the frothing is restricted, and so long as the flame is shielded and not tampered with, there is no sucking back. We are now using with uniform success, an apparatus which can easily be constructed in any laboratory, using the following or similar materials:

- 1 small iron support, 18 inches high rectangular base
- 1 iron retort ring, 5 inch diameter
- 1 iron retort ring, 3 inch diameter
- 2 burette clamps
- 1 five inch wire gauze square
- 1 micro burner
- 1 empty ether can (1 pound size)
- 1 round pyrex flask, 100 cc capacity, 15 mm neck
- 1 No. 0 rubber stopper
- 1 piece glass tubing, 7 mm inside diameter, 24 inches long
- 1 Folin blood sugar tube

The center of the 7 mm glass tubing is heated over a flat wing burner and drawn out about eighteen to twenty-four inches, then reheated and

The motors used by the author were supplied by the Folsom Auto Specialty Company of Cleveland, Ohio, which manufactures both suction and pressure types.

†From the National Pathological Laboratory, Chicago.

Received for publication December 10, 1937.

separated at the center in the same manner as capillary pipettes. Each piece is now marked with a file across the constricted portion, six inches from the beginning of the constriction, broken off square, and the edges smoothed off slightly in the flame. Two bends are made as shown in the illustration, the first bend beginning not less than eight inches from the small end. The No. 0 stopper is bored to fit snugly over the large end of the tube.

The bottom is cut out of the ether can, so that when it is inverted the micro-burner can pass through the small opening in the top. The apparatus

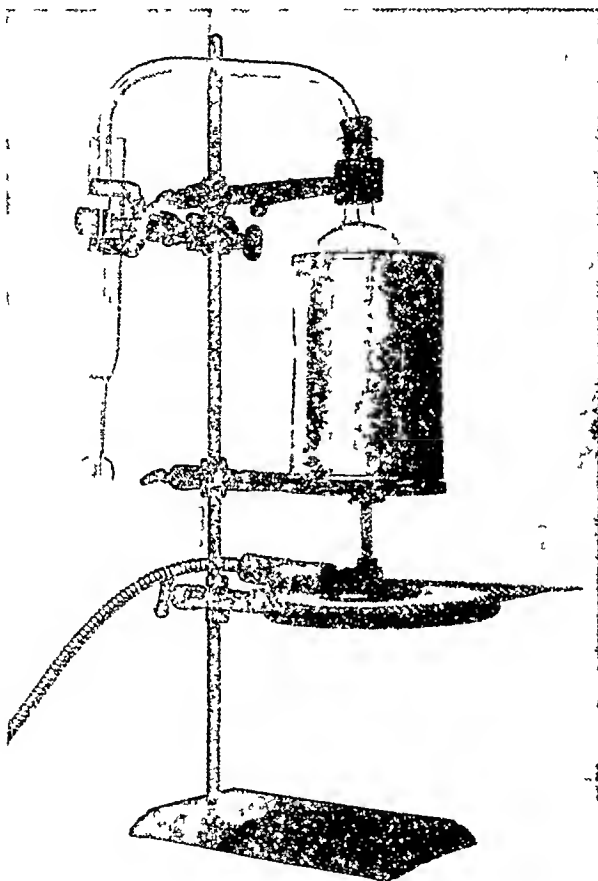


Fig. 1—Apparatus in position ready to begin distillation

is now assembled as shown in Fig. 1. The advantage of using the Folin blood-sugar tube as a receiver is the fact that the steam issuing in a fine stream near the bottom must pass through a column of liquid about an inch and a half in height, insuring more complete absorption of ammonia than when a plain tube of greater diameter is employed. This tube is graduated at 25 cc.

We incubate 5 cc of blood filtrate with 1 cc of urease solution and two drops of buffer mixture in the 100 cc flask, for ten minutes at 40° to 50° C, then add 2 cc of saturated borax solution and one drop of caprylic alcohol (to prevent frothing until steam pressure is generated), adjust the

stopper with bent tube attached, so that the bottom of the constricted portion almost reaches the bottom of the Fohn sugar tube, which contains 2 c.c. of twentieth normal acid and one or two cubic centimeters of water, place the ether can shield around the flask, and insert the micro burner with a low flame (about $1\frac{1}{4}$ inch to tip) through the neck of the can, allowing the tip of the flame to come within about one half inch of the bottom of the flask.

No further attention is required until the liquid in the receiving tube has begun to boil. After this boiling has continued vigorously for at least

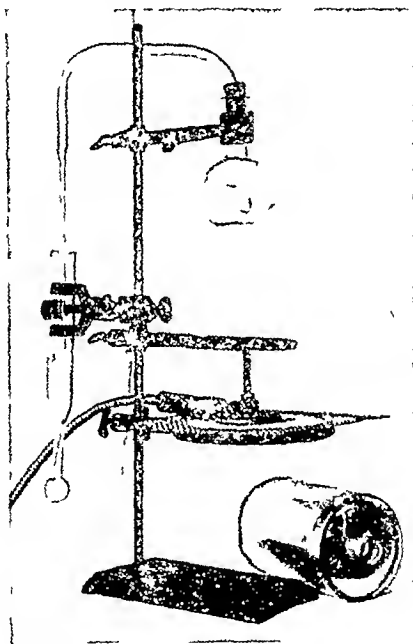


Fig. —Receiving tube drawn down at end of distillation. Ether can removed to show distilling flask.

one minute, or until all traces of the caprylic alcohol (which will be noted condensing in little droplets in the upper portion of the tube) have disappeared, the lower burette clamp is released by loosening the thumb nut and drawn down until the surface of the liquid in the receiver is well below the end of the constricted tube the boiling is allowed to continue one minute longer, and the gas turned off.

The receiving tube is now removed and a little distilled water added allowing room for $2\frac{1}{2}$ c.c. of Nessler's solution which is added as soon as the contents are cooled and the volume made up to the 25 c.c. mark.

THE VARIABLE PARTIAL SOLUBILITY OF BASIC FUCHSIN IN ALCOHOL*

By H. W. WADE, M.D., MANILA, P. I.

A STOCK alcoholic solution of basic fuchsin is employed in one of the two common methods of preparing the carbol-fuchsin stain. The method referred to is to be preferred as decidedly the more convenient, provided it will give reasonably uniform results. The fact is that, as usually prepared, the stock solution is not a dependable factor as regards concentration. Variations may be so great that they may very well affect the accuracy of findings. In any case, such irregularities, beyond those that are unavoidable in the use of dyes as now sold, are certainly not desirable.

There is no definite standard for the stock dye solution, though it is usually called "saturated." Most writers pay no special attention to its preparation, or its final concentration. A belief exists that the saturation point of fuchsin in 95 per cent alcohol is 3 per cent,¹ and Eyre² directs that 3.5 grams be used per cent, with subsequent filtering. Very different is a method for preparing saturated dye solutions in general by which, to begin with, a container is one-quarter filled with the dry substance and then filled with alcohol.³ When tested in a 100 c.c. cylinder this method took 15.2 grams of fuchsin and 88 c.c. of alcohol.

Undoubtedly, the usual procedure for preparing the stock solution is simply to add dye "in excess" to the desired quantity of alcohol. This involves an assumption that solution is complete, or practically so, until a saturation point is reached. In so far as the lot of fuchsin in our stock† is typical of this dye in general, this assumption is erroneous. This fact (or, if it be not constant, this possibility), however, well known to dye technologists, is not available to the medical laboratory worker in the texts commonly used by him.

EXPERIMENTS

Several systematic experiments on the matter were made following an observation that two stock alcoholic solutions prepared by the ordinary "substance-in-excess" method differed grossly in apparent density. Different grades of alcohol (absolute, 95 per cent and 80 per cent) were used in the tests, a preliminary one having shown that solubility varied considerably with the strength of the alcohol.

The various solutions were prepared in rubber-stoppered test tubes, using in each instance 10 c.c. of alcohol. They were repeatedly agitated and kept

*From the Culein Leper Colony, Philippine Health Service.

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†The findings here reported were obtained with a lot of dye manufactured by the Coleman and Bell Company (Lot No. 780, labelled "Pure Crystals" and certified by the manufacturer to be 99.8 per cent soluble in water and 99.9 per cent in alcohol) and supplied by the Arthur H. Thomas Company, Philadelphia. It is entirely satisfactory in use both in staining and in the Endo medium.

slanted to obtain maximum exposure to the solvent. On the following day 5 cc of the fluid was removed for drying usually by pipetting after centrifuging. Usually the solutions were effected at room temperature which ranged from 25 (night) to 32 to 34 (day) but in observing crystallization one extensive set was heated to 57° C and then chilled before sampling they were again warmed to about 40° C.

The results obtained in grams of substance recovered per 100 cc of actual solution are given in Table I and the curves derived from these figures in Fig 1. The curves are straightened somewhat the figures actually obtained reflect the influence of the small quantities used among other things but the inaccuracies are unimportant.

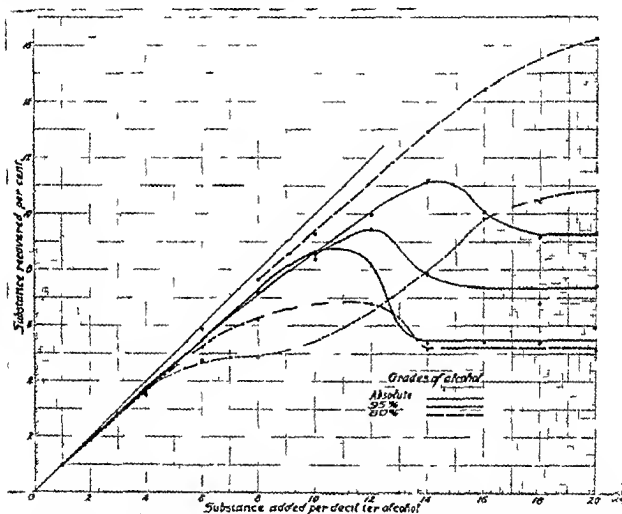


Fig 1—Solution curves of the lot of basic fuchsin tested in absolute 95 per cent and 80 per cent alcohols. The heavier lines (including the single 80 per cent curve) are of the room temperature tests the lighter ones the heated tests.

DISCUSSION OF RESULTS

In none of the proportions used was the dye completely dissolved. Even with as little as 1 gram per 100 cc of 95 per cent alcohol there was some residue and with 2 or 3 grams this was sufficient perhaps to satisfy an inexperienced person that the solution was saturated. This was far from the case. In the proportion of 10 grams of substance to 100 cc of alcohol the concentration of substance (or substances) dissolved amounted to about 85 per cent. The idea that 3 per cent is the concentration of saturation of basic fuchsin in alcohol is at least sometimes far from correct.

The reason for the variable partial solubility shown is not clear. The

TABLE I

CONCENTRATION OF ALCOHOLIC SOLUTIONS OF BASIC FUCHSIN

GRAMS DYE PER CENT ALCOHOL	STRENGTH OF ALCOHOL AND TEMPERATURE					
	ABSOLUTE		95 PER CENT			80 PER CENT
	ROOM	HEATED	ROOM	HEATED		
				(1)	(2)	
1	0.99	1.00	0.96	0.94	—	—
2	1.85	1.90	1.88	1.80	—	—
3	2.80	2.86	2.86	2.84	—	—
4	3.60	3.78	3.50	3.74	—	—
6	5.24	4.72	4.74	5.88	—	—
8	6.20	4.86	7.20	6.76	—	7.62
10	6.44	5.62	8.60 (a)	8.38	8.52	9.26
12	6.80	6.78	8.15 (b)	9.96	9.44	10.62
14	5.40	7.82	5.18	11.16	7.86	12.04
16	5.04	9.80	5.38	10.08	7.66	14.42
18	5.40	10.45	5.38	9.18	6.80	15.32
20	5.14	10.72	5.94 (c)	9.28	7.40	16.24

(a) Average of 2 findings 8.80 and 8.40

(b) Average of 2 findings 8.62 and 7.68

(c) In another test 6.74

Stains Commission points out⁴ that variations in the inert materials used to dilute dye powders affect the amount of dye that will dissolve, wherefore they no longer recommend the use of "saturated solutions" in formulas. But this refers to differences in the maximum solution-concentrations of different lots of dye, not to the condition here discussed. If it be that the relatively small amount of diluent present in one or two grams prevents complete solution when with ten grams total concentration of 8.5 grams is obtained, this indicates marked sensitiveness on the part of some fraction of the dye.

It may be that this is the explanation, and that the curve is due to gradually increasing effect of a mixed dye-diluent solution on the more sensitive portion. It is recognized that basic fuchsin as marketed is a more or less variable mixture of rosanilin homologues, though it would seem that the number of fractions that should be present in any case is not large, indeed, a given lot may be a single chemical entity, as the monomethylated compound (rosanilin), or that with three methyl radicals (new fuchsin).⁵ It would be of interest to compare the solubilities of lots of dye of known constitution. It may be noted that staining tests made to compare undissolved and dissolved fractions showed no important difference.

In most of the series a crystalline slush (*bré*) formed at and beyond a certain point of dye concentration. The crystalline mass was often so bulky as to cause actual solidification. The fluids recovered after crystallization appeared to be truly saturated (flattened curves), though they contained considerably less substance than those below the crystallization-point. Crystallization is apparently not dependent on ordinary supersaturation, it occurs spontaneously, at rising room temperatures, in the presence of an abundance of undissolved dye. It has been suggested to me that in this process alcohol may have been taken up as alcohol of crystallization. It seems uncertain whether the crystals are of dye or of its diluent. However, this is somewhat foreign to the interests of the user of biologic stains.

A feature to be mentioned in passing is that, though the solubility of the dye in pure water was very low (much below 1 per cent), increased water content of the alcohol, within the limits used, greatly increased its capacity to take up the dye. This is striking for 80 per cent alcohol, and it was expected that a 10 per cent stock solution in it would be satisfactory for actual use, but staining solutions made from it appeared turbid and they stained the bacilli (of leprosy) an unattractive dull red. Another feature is that the sets that had been moderately heated attained (and retained after crystallization when this occurred) higher concentrations than the others. However, this obtained only in the tubes containing the larger quantities of dye, larger than those required for staining purposes.

It is evident that stock alcoholic solutions should be prepared with a weighed quantity of dye. Ten grams per 100 cc of 95 per cent alcohol gave fairly uniform results in four tests the concentrations ranging from 8.38 to 8.80, with an average of 8.54 per cent. This concentration is such that carbol fuchsin made by adding 10 cc of this solution to 90 cc of carbolic solution is probably about equal in dye content to that made by adding 1 gram of the same powder to 100 cc of the carbol water plus 10 cc of alcohol and filtering off the undissolved residue. This stock solution has in the past very or so proved entirely satisfactory. In the interest of uniformity it is probably best that the residue be not allowed to accumulate in the stock bottle.

SUMMARY AND CONCLUSIONS

In solubility tests a good commercial sample of basic fuchsin has not gone completely into solution in amounts below the limits of solubility. There is an undissolved residue even in the lower suspension concentrations used. There is, therefore, no definite saturation point in the usual sense of this term. The residues are not uniformly proportional to the amounts of substance used but tend to increase in proportion.

Other features observed are that sooner or later in a test series spontaneous crystallization occurs, that moderate heating causes a distinct increase in the amounts dissolved and held in solution and that though the solubility of the dye in water is low solubility in alcohol increases with its water content. However, these features need not concern the user of the dye in staining.

The main observations, which recent tests with certain other dyes show to be not unique constitute a further reason for abandoning the use of "saturated" solutions made by the substance in excess method. Of the dye tested, solutions made from 10 grams per 100 cc of 95 per cent alcohol which are of approximately 8.5 per cent concentration, have proved entirely satisfactory for making carbol fuchsin stain.

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TWO METHODS FOR THE EOSINOPHILE COUNT IN THE COUNTING CHAMBER FOR ROUTINE WORK*

BY AKIRA SATO, M D, SENDAI, JAPAN

THE differentiation of eosinophiles is, of course, very important in routine blood counts. One may thus be led to suspect infestation with animal parasites, in spite of negative stool findings.

This paper deals with two methods for routine use. The first is for the eosinophile count only. The second is for the recognition of eosinophiles in the counting chamber when stained by peroxidase reaction (copper method). With a little practice one will be able to estimate the number of eosinophiles while making a differential count.

1 Method for Eosinophiles Only—This method is not original, but only a slight modification of the excellent Dunger Method¹. Our experience is that the stain made according to his directions lasted only one or two weeks, after which it became turbid.

PROCEDURE

Two solutions are required

Solution I One per cent water soluble eosin
(Grubler) 20 c c

Solution II (for Eosinophile)
Acetone 80 c c
Distilled water up to 400 c c

Before using, mix one part of Solution I and four parts of Solution II. Draw the blood to the mark, then fill with the solution up to the mark II. The fluid is then introduced into the counting chamber. The concentration of eosin is very low compared with that in the original method, but it is very satisfactory.

2 Eosinophile Count by the Peroxidase Reaction—The second method was not originally designed for counting eosinophiles, but to differentiate

*From the Department of Pediatrics Faculty of Medicine Tohoku Imperial University Sendai Japan.

lymphatic and myeloid cells in the counting chamber. However when one has acquired some practice with the method of Sato and Shoji one will be able to count monocytes separately from the other myeloid elements, because they will appear red with the low power objective, although they contain blue granules distinctly recognizable with the medium power objective. The eosinophiles are a dark blue among the lighter blue neutrophils when seen with the low power objective. In Table I is given the eosinophile counts by this method and also by method I described above.

The results of the two methods give good agreement. The second method thus gives a rough estimate of the eosinophile count, with no extra time or trouble. Tokue in our laboratory will report further in the near future.

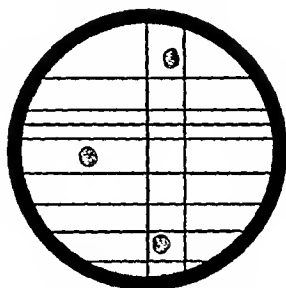


Fig 1—The eosinophile count in counting chamber

TABLE I

	INFANTS	COUNTING CHAMBER PEROXIDASE METHOD		MODIFIED DUNGER METHOD
		TOTAL LEUCOCYTE COUNT	SUSPECTED EOSINOPHILE CELLS IN PERCENTAGE	EOSINOPHILE CELLS IN PERCENTAGE
Case I	(Saijo)	10900	4	4
Case II	(Kanemoto)	8000	4	4
Case III	(Kikuchi)	5080	16	15
Case IV	(Okawa)	3630	4	4
Case V	(Kaneda)	7800	14	13

SUMMARY

Two methods for counting eosinophiles are described. One is a modification of the Dunger method with permanent reagents. The second is the counting chamber method of the peroxidase reaction (copper method) in which the eosinophiles are estimated in the differential count. The results of the two methods agree.

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COUNTING CHAMBER PEROXIDASE METHOD FOR BLOOD SIMULTANEOUS RAPID DIFFERENTIAL LEUCOCYTE COUNT AND TOTAL LEUCOCYTE COUNT*

BY AKIRA SATO, AND KENJI SHOJI, SENDAI, JAPAN

FOR more than two years since the development of the new peroxidase reaction of Sato and Sekiya,¹ we have endeavored to adopt it for use in the counting chamber. The purpose was, first, to shorten the time for routine examination of blood cells and, second, to simplify the technic so that it could be done by a technician without special training. This objective has been attained in the following method.

PROCEDURE

The myeloid leucocytes are stained blue by the peroxidase reaction (cop per method) and the lymphocytes red by a counter stain. The sum of these two counts gives the total white count.

Apparatus required 1 A leucocyte pipette 2 A counting chamber

Solution A —

Aqueous solution of copper sulphate, 1 per cent	-	-	-	90 cc
Acetic acid, 30 per cent	-	-	-	1 cc
Glycerin	-	-	-	2 cc
Distilled water up to	-	-	-	300 cc
Filter				

Solution B—Rub 0.5 gm of benzidine, pur Merck (or benzidine base Merck) in a mortar and add distilled water up to 200 cc and filter. To the filtrate add 4 drops of 3 per cent hydrogen peroxide. It should be preserved in a brown bottle.

Solution C—This is a one per cent aqueous solution of safranin. Filter through good filter paper. Before using, pour 2 cc of Solution A in a clean watch glass and add two drops of Solution C. Take 2 to 3 cc of Solution B in another watch glass.

Draw blood into the pipette up to the 0.3 mark (not 0.5) and after rapidly wiping the end clean, immerse it at once into Solution A plus safranin. Draw up this solution until the mixture fills the lower third or fourth of the bulb of the pipette. Hold a finger over the end and rotate the pipette on its long axis. This causes mixing and avoids the formation of air bubbles. Wait four minutes and then fill to the 1.0 mark with Solution B. Insure thorough mixing by rotation. The fluid is then introduced into the counting chamber.

*From the Department of Pediatrics, Faculty of Medicine, Tohoku Imperial University, Sendai, Japan.

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RESULTS

- 1 Myeloid leucocytes are stained greenish blue Monocytes appear faintly red with distinct blue granules
- 2 Lymphocytes are stained distinctly red without any shade of blue or green (Fig 1)

REMARKS

- 1 Differentiation of myeloid and lymphatic leucocytes is easy All it requires is the ability to distinguish between red and blue The difference does not depend upon morphologic details, but upon specific biologic relations of the leucocytes
- 2 The differential and total cell counts are made simultaneously and require no more time than the usual white count
- 3 If by accident some blood is lost or fluid drawn beyond the 110 mark or an air bubble is introduced though the total count is not correct the differential count is still accurate

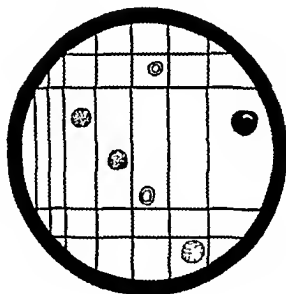


Fig 1

PRECAUTIONS

One who tries the present method may wonder why it took so long to develop it after success had been attained with the peroxidase method. Although the technique is simple one may fail unless certain precautions are taken.

- 1 It is essential that four minutes elapse after drawing up Solution B plus safranin. We utilize this time in going from the ward (where the blood is drawn and the first solution added) to the laboratory (where the second solution is added and the count made). Any time in excess of four minutes is satisfactory.

- 2 Fluids should never be withdrawn directly from the bottles, but always from clean watch glasses. Solutions A and B should never be mixed beforehand nor Solution B and safranin.

- 3 The mixture in the pipette when filled to the mark 110 should appear transparent and light pink, not blue. If both conditions are not fulfilled the stain invariably fails.

4 Blood should not be drawn up higher than 0.3. Beginners fail when they take more, but those with experience never fail even with 0.5. With the smaller amount one never fails to carry all the blood from the capillary to the bulb.

5 Beginners usually draw up too much of Solution A plus C. They should aim to fill the bulb only one-fourth full.

6 Solution A plus C should be drawn up immediately after the introduction of the blood.

7 In mixing, some care must be exercised to prevent the formation of air bubbles. If this has occurred the differential count can still be made.

8 The present method is applicable for human blood only. The treatment of blood of other species will be described in the near future.

9 The microscopic field should show no blue or green fragments. If they are abundant it is a sign of failure.

10 Alcohol and ether should be removed from the cleaned pipette.

11 The low power field should show distinct blue cells. If blue granules can be seen only with the high dry objective, the stain has failed.

12 Solution A keeps indefinitely.

13 Solution B may seem to deteriorate soon, but it keeps for some months in a brown bottle or one year in the dark.

REMARKS

1 After we had succeeded with the peroxidase method given above, we tried the oxidase reaction of Winkler and Schultze² with the counting chamber technique and were able to attain success. However, even with freshly prepared reagents, the microscopic field shows much colored detritus. We hope to publish a note on this method soon.

2 The method described has been used in our laboratory for some time. By its use was discovered the first case of agranulocytosis ever reported in Japan.³ The case was one of apparent sepsis. The field showed no blue (peroxidase-positive) cells.

3 The application of the method to cerebrospinal fluid will be published by Shoji.

SUMMARY

The peroxidase reaction (copper method) of Sato and Sekiya shows a clear differentiation between lymphatic and myeloid leucocytes in the blood smear. The present paper describes the adaptation of that method to the counting chamber.

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A NEW ETHERIZING BOTTLE FOR EXPERIMENTAL WORK*

By D L JACKSON, PH D M D CINCINNATI, OHIO

I HAVE experimented with a considerable number of etherizing bottles or devices, but after a year's use, the valve and jar shown in Fig 1 have been found more satisfactory than any other heretofore used

The device consists of two parts Fig 1 shows the complete device as assembled and ready for use Fig 2 shows the metal parts removed from

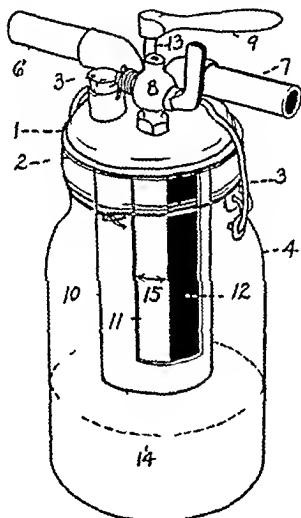


Fig 1

the standard, one quart size fruit jar (4) which is filled with ether only up to the dotted circle (14) The breathing tube (6) or (7) is connected to the tracheal cannula by one half inch size rubber tubing, and the respired air passes back and forth through the tubes and also through the lumen of the double metal, fenestrated cylinders (10 and 11) This inner cylinder (11) is movable and can be rotated inside the outer cylinder (10) by means of the handle (9) There are two large vertical windows in each cylinder By turning the handle (9) these windows can be rotated around so that the two win

*From the Department of Pharmacology University of Cincinnati Medical School Cincinnati Ohio

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dows on each side will exactly coincide with each other (thus permitting the ether vapors to pass into the inner cylinder and hence into the respiratory current through the breathing tubes), or the inner cylinder can be turned back so that the outer windows will be partially or wholly closed off by the nonfenestrated parts of the inner cylinder (when only a little or no ether vapor will enter the inner cylinder and the animal will breathe back and forth through the inner cylinder but will get only a little or no ether). By turning the handle back and forth the two double windows can be opened a little (or widely) as indicated by the double arrows at (15). Thus any desired degree of anesthesia can be obtained and in practice a perfectly regular and even anesthesia is easily maintained for hours at a time. The dark space (12) represents the opening into the inner cylinder when the two sets of double windows are about half closed off.

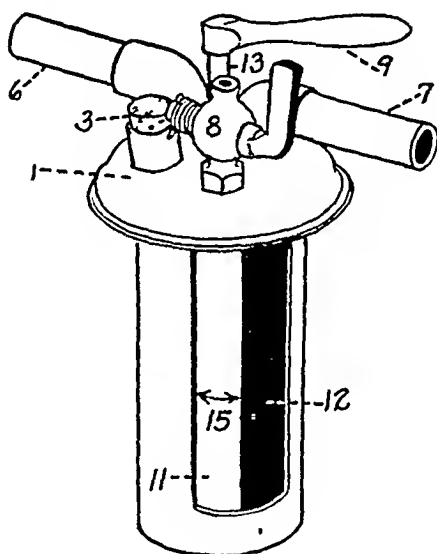


Fig. 2

The spun metal top (1) carries the breathing tubes (6 and 7) and a tubulature closed by the cork (3). Ether can be poured into the jar through the tubulature while the device is in use. Through the faucet (8) ethyl chloride, carbon dioxide, etc., can be run in through a one-fourth inch rubber tube. The metal top (1) is held on to the jar by the regular wire device (3) which is used to hold on the lid when the jar is used for ordinary purposes. A rubber gasket (2) serves to make the connection air-tight. The rod (13) connects the handle with the inner cylinder.

Artificial respiration can be given through the device without changing any connections or without varying the resistance of the air pressure (and the degree of inflation of the lungs). In fact it was largely to meet this latter requirement that the device was originally designed. For when the chest is open and artificial respiration is being given by means of the usual devices, all the records which are being taken will be disturbed if the amount of ether being given is changed. A T-tube-tracheal cannula should be used and a short

piece of rubber tubing carrying a small screw clamp is placed on the open end of the cannula to secure proper adjustment for the required lung inflation with the given current of air. The device is made by the Max Woehner and Son Co., Cincinnati.

A NEW MYOCARDIOGRAPH*

By D. E. JACKSON, PH.D., M.D., CINCINNATI, OHIO

THE myocardigraph here shown was designed especially for dogs but may be used for cats or rabbits. The animal is anesthetized and the chest is opened widely, exactly in the midline in the usual fashion. The pericardium is opened longitudinally and each side is attached to the chest wall by one or two stitches so as to hold the heart suspended in a kind of "hammock," but in approximately its regular normal position. The long horizontal arm of the device [i.e. the section between (28) and (31)] is brought into position exactly above the opening in the chest the distance (32) to (31) being directly over the heart and almost in immediate contact. The hinge joint (28) is directed toward the head of the animal and the end of the sternum at the base of the neck must be opened wide enough to permit the section (28) to (26) to move with perfect freedom and without touching any obstruction whatever. This permits absolutely free movement in any direction of the distal part of the horizontal arm where it attaches to the heart.

The apparatus as a whole is grouped on to a base plate (3) which is held fast to a stand (4) by means of the double clamp (1) which firmly holds the supporting rod (2) of the base plate. A horizontal hole is drilled through the base plate and into the right hand end of this hole the tube (14) is soldered. A small pulley (5) is placed at the lower edge of the left hand end of the hole drilled through the base plate. The thread (8), which moves the lever (6) passes over the pulley (5) and through tube (14) and tube (16) and over pulley (18) down through tube (20), around pulley (24) through tube (33) and out to the point (32) at which location the thread *when the device is in use*, is attached to the upper part of the ventricle by one or two small stitches. This attachment is made before the metal bow (30) is sewed to the lower end of the ventricle through a small hole in the metal at point (31).

Thus when the heart beats the contraction of the ventricle will cause a shortening of the distance between the attachment of the thread (32) and the attachment of the metal at (31). The metal arm moves freely in all directions without moving the writing lever but pulling on the thread (which runs over the pulleys) causes immediate movement of the metal writing lever (6).

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Since the horizontal metal arm [(28) to (31)] can move freely without affecting the tracing the respiratory movements of the lungs, etc., will not disturb the record

The device has hinge joints at (19) and (28) Swivel joints for the tubes are placed at (27), (29), and (17) Longitudinal as well as circular adjustments can also be made at the locking collars (12) and (15) and (21)

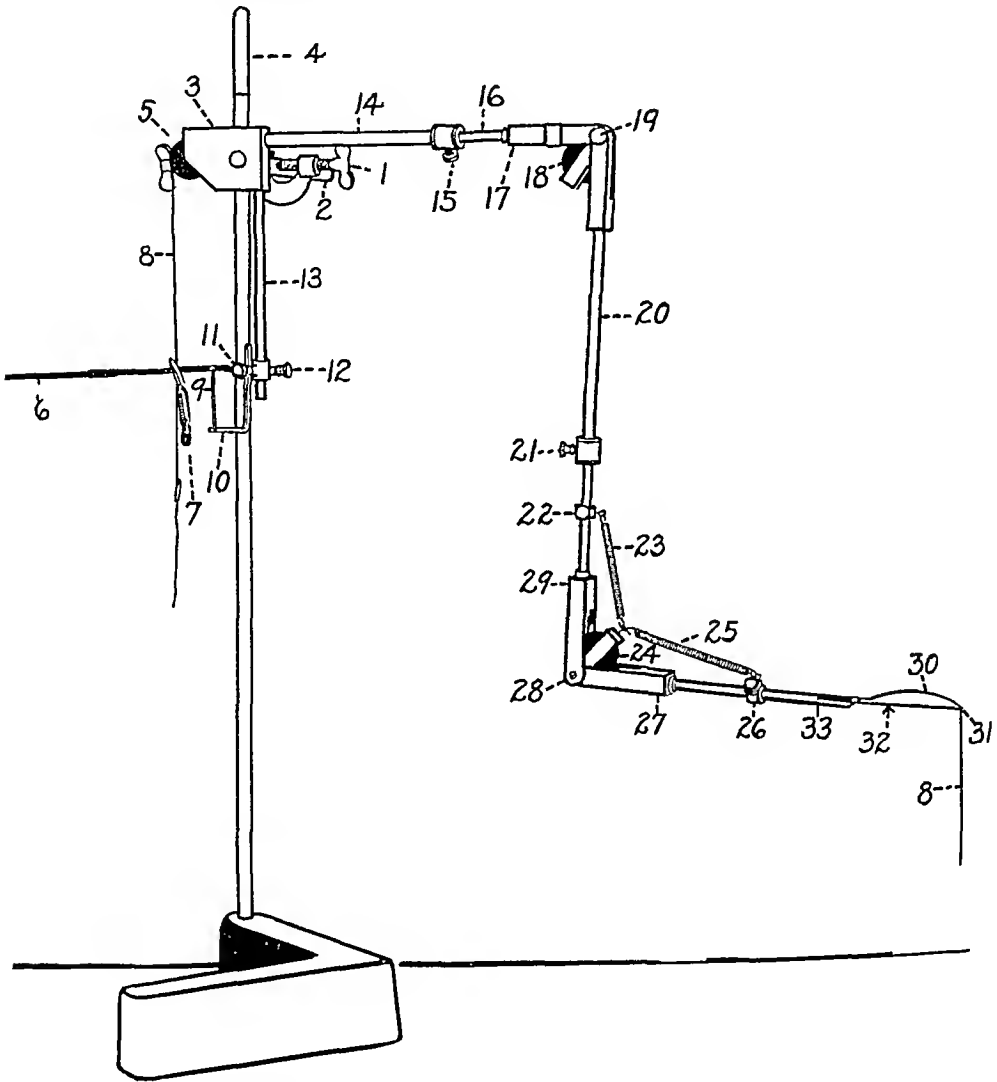


Fig 1

The supporting springs (23), (25) are adjusted and held in position by the setscrew-adjusting collars (22) and (26)

Perpendicular and circular adjustments of the writing lever can be made by moving the locking-collar and set screw (12) Tension on the spring (9) is regulated by adjusting the bar (10) which is held by a set screw (11) The thread (8) is best attached to the metal writing lever by a bull dog clamp (7) which can be adjusted easily and quickly

I have found this myocardiograph to be sufficiently sensitive and accurate to bring out a number of points about cardiac activity which I had not appreciated before and which I believe no other instrument has shown. The device is made of brass and is nickel plated. It can be obtained from the Max Woehner and Son Company, Cincinnati, Ohio.

STRING SALOL TEST FOR INDICATING PRESENCE OF THE BUCKET IN THE DUODENUM*

By MOSES EINHORN, M.D., NEW YORK

THOSE physicians who find it necessary to perform frequent biliary drainage are well aware of the lack of a suitable method for indicating that the bucket has entered the duodenum. My experience in this respect has taught me that most of the methods and devices at present in use for this purpose are either impracticable or unreliable. Thus, I have found that the most reliable test is by far the most impractical one. I refer to the practice of fluoroscopic examination of the patient in order to ascertain the presence of the peculiar S shaped formation of the tube which indicates that the bucket has entered the duodenum. The reliability of this test is however offset by its impracticability, for it entails changing the patient's position and this is contrary to the technique of biliary drainage which lays so much stress upon the proper position of the patient. Even were we able to disregard this disadvantage, the fact that so few physicians have fluoroscopes would still prevent this test from being used extensively. Being forced to discard this test because it is impractical, the physician reverts to the usual procedure of using the appearance of bile to indicate that the bucket has entered the duodenum. When this test is employed, we find that although it is to be recommended for its simplicity we cannot place too much credence in the results it produces because of their uncertainty. This test requires the physician to investigate two possibilities, i.e. the bile may be acid or alkaline in its reaction. If the former, it is bile which has been regurgitated into the stomach, if the latter it comes from the duodenum. However the physician cannot always depend upon these reactions to inform him whether the bucket is in the stomach or in the duodenum. It often happens that there remains in the tube sufficient acid from the previous stomach drainage to create an acid reaction in the bile from the duodenum which is passing through the tube. The physician, upon testing the bile and discovering that it has an acid reaction is often led to believe that the bucket is still in the stomach, whereas, as a matter of fact it has already entered the duodenum. These few illustrations are sufficient I believe to show the need of an apparatus which will eliminate the difficulties described above.

In the course of my work upon the Analytic Bucket,¹ I had occasion to

make use of the principle often used in pharmacologic preparations when, in order to prevent the preparation from being dissolved in the stomach, a coating of salol (phenyl salicylate) dissolvable only in the alkaline medium of the duodenum is applied to the preparation. It occurred to me that this principle might be used to advantage in solving the problem which confronts us. Accordingly, I have devised the following apparatus which is based upon my New Tip for Gastroduodenal Tubes,² the principal characteristics of which are its three-part composition, capsule shape and spiral arrangement. The bottom portion of this Tip was cut off and so modified that it could be connected with the rest of the Tip by means of a screw and thread arrangement. A small piece of brass in the form of a scoop was soldered onto the screw in



Fig 1

the severed portion of the Tip in such fashion that it projected within the spiral chamber when the bucket was assembled (see Fig 1). By means of a thin wire with a hooked end, a length of surgeon's thread is drawn through the tube and attached to the scoop in the following manner. The end of the thread is dipped in phenolphthalein, placed in the scoop and covered with a few drops of melted salol. The salol cools rapidly and upon hardening holds the thread strongly in place. The thread cannot be withdrawn until the salol has been dissolved and, because of the peculiar properties of salol, this can take place only in the duodenum.

This apparatus does not involve any changes in the usual drainage technique.

During the course of the drainage, the physician should tug gently upon the end of the string which emerges from the tube. If the bucket has entered

the duodenum the action of the alkaline medium will have dissolved the salol and caused the end of the string to turn a scarlet color. The physician then will be able to withdraw the string without disturbing the position of the bucket. If the string cannot be withdrawn, it is an indication that the salol has not come in contact with the alkaline medium of the duodenum and consequently informs the physician that the bucket has not yet entered that organ.

The objection may be raised that this device is not reliable in those cases where there is regurgitation of bile into the stomach, for then, it may be argued, the bile in the stomach will dissolve the salol and permit the string to be withdrawn even though the bucket has not entered the duodenum. This possibility did not escape me when I was experimenting with this device, but as a result of the various tests which I have made, I believe that I can safely say that the objection is not a valid one. In the first place the amount of bile which is regurgitated into the stomach is usually insufficient to neutralize the acid medium present there. Even were we to grant that regurgitation of bile will create an alkaline medium in the stomach as for example in those cases of true *Achylia Gastrica* where there is no acid medium in the stomach nevertheless, the peculiar properties of salol are such that it is dissolvable only under a steady stream of biliary and pancreatic secretions particularly the latter, and that is a condition which we can never find in the stomach.

Although I offer this method to my colleagues, confident in the belief that the basic principle is a sound one I nevertheless realize that there is still room for research and investigation upon several of the minor aspects of the solution which I have advanced. Thus I have found that it requires much patience and effort to draw the thread through the tube and since this operation must be repeated before each drainage I believe that a more efficient method of accomplishing this would add greatly to the usefulness of this device. We must also learn just how thick a coating of salol should be put upon the strips and obtain more information upon the question as to how much time is required for the salol to be dissolved in the alkaline medium of the duodenum.

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A METHOD FOR READING THE KAHN PRECIPITIN TEST FOR SYPHILIS THE EFFECT OF CENTRIFUGATION*

BY S L LEIBOFF, NEW YORK CITY

OF THE various precipitin tests devised for the diagnosis of syphilis, the Kahn¹ test has met with universal favor. The accuracy of the Kahn test has been fully substantiated by many subsequent investigators. The success of the test rests upon the fact that Kahn recognized the importance of using the ingredients of the test in a highly concentrated form. He adjusted the serum, antigen, and normal saline in proportions that assure the highest degree of concentration possible.

While the Kahn is the most ideal precipitin test for the diagnosis of syphilis, it, nevertheless, has a great drawback in the optical difficulty encountered in reading weakly positive sera, due to the low visibility of the very fine particles that constitute the reaction.

A number of devices have been suggested to overcome this difficulty. Kahn recommends that the reading should be made in front of a partly shaded window against a darkened background, and that in all doubtful tests each tube should be removed from the rack and carefully examined under the most favorable conditions of lighting. Recently, Hopkins and Rockstraw² have devised a special illuminating device with the use of artificial standard light to render readings more uniform.

While these devices are helpful, they do not completely eliminate the difficulty, and one has to rely chiefly upon experience and personal equation in reading weakly positive sera. As Kahn³ recently stated, only especially trained and experienced workers should be considered qualified to perform the test.

In attempting to simplify the reading of weakly positive sera in the Kahn test, it became evident that the difficulty in distinguishing the precipitate in such sera was not due solely to the smallness of the particles, but to other interfering substances. While some sera are particularly cloudy, all of them, even the very clearest, show some degree of opalescence due to the high content of colloidal material. This, together with the highly opalescent antigen, obscures minute precipitates and renders heavier precipitates more difficult of recognition.

We may safely assume that the amount of precipitated substance, particularly in weakly positive sera, is comparatively small in proportion to the rest of colloidal material normally present. Thus, were it possible to remove all extraneous matter, the particles of the precipitate would be far more visible. This can be readily accomplished by means of centrifugation.

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A preliminary trial with a few syphilitic and nonsyphilitic sera was convincing of the practicability of such a procedure. Accordingly this was tested out as follows. Twelve sera were used in the experiment, of which six were positive and six were negative, as shown by the Wassermann test. Of the positive sera four were 4+, one was 2+ and one 1+-. The Kahn test was performed on the twelve sera according to the standard technic. In reading the results no difficulty was encountered with the four 4+, nor with five of the negative sera. Some difficulty was met with the reading of the 2+ serum, but, this was, most likely, due to a very limited experience with the Kahn test. On viewing this serum through a magnifying lens the particles could be seen very easily. With the 1+- and with one of the negative sera in conclusive results were obtained even with the aid of a lens.

All the tubes were then centrifuged at a moderate speed for five minutes. It then became apparent that all the positive sera including the 1+- serum, had a moderately large film of opaque material deposited at the bottom of the tubes, the amount of this deposited precipitate apparently being proportionate to the strength of the sera. None of the six negative sera showed a deposited precipitate. The supernatant fluid was then poured off by inverting the tubes. After adding half of one c.c. of saline to each tube they were shaken vigorously for about half a minute in order to break up the film and were reexamined. The results obtained are shown in Table I.

None of the tubes containing the negative sera showed particles when examined with the naked eye or with a lens. Most of them appeared almost water clear, while some showed a slight opalescence when viewed against a dark background. This was doubtless due to traces of material remaining in the tubes, which, of course is unavoidable. This very faint opalescence, however, could in no way be confused with the precipitated particles in the positive sera.

All the tubes containing the 4+ sera showed great numbers of comparatively large particles suspended in a clear medium. These could easily be seen from a distance of about three feet. The particles in the front tubes appeared somewhat larger and more numerous than in the back tubes. Some of the 4+ sera showed larger particles than others, this being due, most likely, to the varying strength of the different sera.

The 2+ serum showed many particles in the first two tubes. These particles were smaller than any of the 4+ sera, and were fewer in number. The third tube looked doubtful to the naked eye, but through the lens showed distinct minute particles.

The 1+- serum showed very minute particles in the first tube only. The second tube looked doubtful even through the lens, and the third tube was definitely free of particles.

Very definite results were obtained with a hundred more negative and 40 positive sera.

While the number of sera tested is, admittedly, very small, yet the results are very convincing, and it is hoped that the use of the findings here

set forth as an adjunct to the widely accepted Kahn test, would go far to clear up the discussion as to the relative value of this precipitin test and the Wassermann test in weakly positive sera

TABLE I

EFFECT OF CENTRIFUGATION UPON THE PRECIPITATE FORMED IN THE KAHN TEST

SERUM NO	WASS TEST	KAHN TEST					
		TUBE 1 (05 CC ANT)		TUBE 2 (025 CC ANT)		TUBE 3 (0125 CC ANT)	
		BEFORE CENTR	AFTER CENTR	BEFORE CENTR	AFTER CENTR	BEFORE CENTR	AFTER CENTR
1	++++	++++	++++	++++	++++	++++	++++
2	++++	++++	++++	++++	++++	++++	++++
3	++++	++++	++++	++++	++++	++++	++++
4	++++	++++	++++	++++	++++	++++	++++
5	++	++	++++	+	++	-	+
6	+-	?	++	?	?	?	-
7	-	o	-	?	-	?	-
8	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-

SUMMARY

It was found that the difficulty encountered in reading weakly positive syphilitic sera in the Kahn precipitin test for the diagnosis of syphilis, is not due entirely to the number and size of the particles formed, but to the extraneous substances which obscure the particles. Thus, the reading of weakly positive sera may be made much easier by centrifuging the precipitates, and resuspending them in clear saline.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D. ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

IVY POISONING Observations on the Use of a Modified Extract from *Toxicodendron Radicans* Spaul W O and Cooke R A Jour Immunol, xiii No 2 93

The active principle of poison ivy *Toxicodendron radicans* retains its full potency indefinitely when kept under water free conditions This is possible by storing under calcium chloride, the fresh green leaves thoroughly dried or by storing the absolute ethyl alcohol extract of such leaves

A satisfactory degree of clinical immunity to ivy poisoning can be developed by the administration in proper amounts of the active principle of *Toxicodendron radicans*, either in the form of oral treatments or hypodermis injections

The injection method of treatment is preferable since the conditions surrounding it can be kept more fully under control than in the case of oral administration

The characteristic lesions of dermatitis venenata can develop upon areas of skin or mucous membrane which have not been in surface contact with the active principle its transmission to the areas involved being through the blood stream or lymphatics from a distinct focus

The development of a clinical degree of immunity as a result of treatment is indicated by the history of improvement and not by any demonstrable change upon test in the reaction of the skin toward the active principle

LABORATORY TECHNIC

CULTURE MEDIUM A Modification of the Kligler Lead Acetate Medium, Bailey S F and Lacy G R Jour Bacteriol 1927 xiii No 3 183

The essential points noted are

1 Phenol red gives more satisfactory results as an indicator than Andrade's indicator which is used in the Kligler medium

2 It is not necessary to add the sterile lead acetate to melted sterile agar tubes but all of the ingredients of the medium can be mixed in bulk at a temperature of 50 C or lower thus preventing flocculation The lead acetate can be added either in crystals or in solution The medium can then be tubed and sterilized by heating in the autoclave at 5 pounds pressure for twenty minutes

3 No damage occurs and no flocculation takes place even though sterilization be accomplished by heating at 15 pounds pressure for fifteen minutes

4 The browning of the lead acetate medium is due to the formation of lead sulphide and in the absence of this medium the production of H₂S by organisms may be determined by suspending in the neck of the culture tubes strips of white filter paper saturated with lead acetate solution

5 The preparation of the medium thus simplified makes it thoroughly practical for use in teaching laboratories and in public health diagnostic laboratories

To each 100 cc are added 1 gm of lactose 0.1 gm of glucose 0.05 gm lead acetate and 5 cc of 0.02 per cent aqueous phenol red the medium having first been cooled to 50 C

The following formula was used throughout as a basis for the medium

Bacto beef extract	5 grams
Pepton (P D)	10 grams
Sodium chloride (B & A)	5 grams
Agar shreds	15 grams
Tap water	1000 cc

The agar was first thoroughly washed in running water and was then heated in the required amount of water, until it was dissolved, after which the other ingredients were added. The reaction was adjusted to P_H 7.4 and the medium was boiled for from five to eight minutes. At the end of the heating, the reaction was readjusted to P_H 7.4 and the sediment allowed to settle to the bottom of the container. The clear supernatant agar was then decanted into another container and was accurately divided into quantities of 100 cc and sterilized by heating at 10 pounds pressure for twenty minutes.

PREGNANCY Postmortem Findings in Ten cases of Toxemia of Pregnancy, Bell, J. W. *Am Jour Obst and Gynec*, 1926, 11, No. 6, 792

In these cases there is little agreement in the liver lesions, which include passive congestion, localized fatty infiltration, acute yellow atrophy, infarction, hemorrhagic necrosis, cellular infiltration (chiefly of portal spaces). These data weaken our belief in any one lesion of the liver being considered essential for toxemia of pregnancy.

RABIES A New Stain for Negri Bodies, Sellers, T. F. *Am Jour Pub Health*, 1927, 17, 1080

Basic fuchsin (saturated absolute methyl alcohol solution)	2.4 cc
Methylene blue (saturated absolute methyl alcohol solution)	15 cc
Methyl alcohol (absolute acetone free)	25 cc

The methylene blue and the methyl alcohol are mixed in a Coplin jar and 2 cc of the saturated fuchsin solution is added. A trial stain is made. Macroscopically the properly stained smear, when held up to the light, should appear reddish violet in the thinner areas shading into purplish blue in the thicker portions. If in the trial stain the thinner parts are bluish, 0.5 cc more fuchsin should be added and another trial made. Two cc of fuchsin solution is nearly always sufficient. The mixed stain seems to improve after twenty-four hours' standing and thereafter keeps indefinitely if protected from evaporation, which tends to make the fuchsin become too dominant. The addition of absolute methyl alcohol to the evaporated stain will usually restore the proper balance to the two dyes. On the basis of the above formula the stain may be prepared in any quantity, and if kept tightly corked will maintain its quality indefinitely.

TECHNIC OF STAINING

Thin smears from the Ammon's horn hippocampus are prepared in the usual way and while still moist with tissue juice are plunged into the stain, removed immediately and rinsed under the tap. The entire process should be completed in less than five seconds. The stained slide may be dried by warming and waving through the air. Blotting is not recommended because the wet smear is very easily wiped from the glass.

The low power two-thirds objective should first be used in examining the stained smear. Thin areas showing numerous large nerve cells well spread out should be spotted and then examined with the oil immersion.

CARCINOMA, "MALIGNANCY INDEX" IN The Significance of the Histological "Malignancy Index" for Prognosis and Treatment of Carcinomata of the Cervix Uteri, Schmitz, H., Hueper, W. and Arnold, L. *Am Jour Roentgen and Radium Therapy*, July, 1926, 11, 1, 30

In this paper, illustrated with 12 microphotographs, the authors describe their method of determining the "malignancy index."

The following factors were used:

1. Special cell type of carcinoma. Each of the four subgroups of carcinomas were used as a basis in this special cell type classification. The cornified spinous cell carcinoma was given a value of 2, uncornified spinous cell carcinoma a value of 4, round cell car-

cinoma a value of 6 and the spindle cell carcinoma a value of 8. The same general scheme was carried through with the glandular carcinomas. The maturity of the majority of cells present was the main factor used to determine this grouping. The more embryonal and anaplastic the component cells the greater is the proliferative power and the higher the potential malignancy.

2 and 3 Irregularities in size and shape of the cells. The average shape or size was studied. If 50 per cent of cells showed irregularity in size and shape then the value 4 was given. If 30 to 40 per cent the value 3. If 20 to 30 per cent the value 2. and if 10 to 20 per cent the value 1.

4 Distinctness in outline of the cells. The cells with distinct and sharp outline were evaluated as 1. If 50 per cent were indefinite in outline the value 2 was given. If 75 per cent the value 3. and if more than 75 per cent had an indefinite outline a value of 4 was given.

5 Functional activity of cells. This was estimated from the presence in the cells of keratin granules mucus etc. If the majority of the cells showed a functional activity the value 1 was given. Minor degrees were evaluated as 2, 3 and 4 respectively. The same relative percentages were used as in the previous standards.

6 and 7 Irregularities in the size and shape of the nuclei of the cells. The size and shape shown by the majority of the nuclei present were taken as the standard size and shape. A deviation of 50 per cent from this standard was evaluated 4. a deviation of 30 to 40 per cent 3. of 20 to 30 per cent as 2. and less than 20 per cent as 1.

8 Staining quality of the nuclei. Cells containing nuclei taking the stain as intensely as the nuclei of lymphocytes were considered hyperchromatic. When 25 per cent of such hyperchromatic nuclei were present the value of 4 was given. 20 to 25 per cent a value of 3. 10 to 20 per cent a value of 2. and 5 to 10 per cent a value of 1.

9 Number of mitoses and prophase. These two were counted separately but considered together as indicative of proliferative properties. Ten fields were counted with oil immersion lens (1000 x magnification) giving an actual count on the mitotic figures on which to base an estimate of proliferative activities of the carcinoma examined. In case 20 or more mitotic figures were seen in the 10 fields a value of 4 was given. 15 to 20 mitoses were evaluated as 3. 10 to 15 as 2. and below 10 as 1.

An examination of the tables in the paper will show how the numerical values of the various factors are put in a certain system which was named malignogram (Hueper) and when added together give a number to which the term histological malignancy index (Hueper) was applied.

In addition to these it is suggested that a study of the factors below would increase the value of the malignogram.

- 1 Special type of carcinoma
- 2 General type of the carcinoma
- 3 Absolute size of the cells
- 4 Irregularities in size of the cells
- 5 Absolute shape of the cells
- 6 Irregularities in shape of the cells
- 7 Distinctness in outlines of the cells
- 8 Staining quality of the cells
- 9 Functional activity of the cells
- 10 Absolute size of the nuclei
- 11 Irregularities in size of the nuclei
- 12 Absolute shape of the nuclei
- 13 Irregularities in shape of the nuclei
- 14 Absolute staining quality of the nuclei
- 15 Irregularities in the staining quality of the nuclei
- 16 Number of mitoses and prophase
- 17 Irregularities in form of the mitoses
- 18 Character of the stroma

- 19 Vascularity of the stroma
- 20 Degree of round cell infiltration
- 21 Character of round cell infiltration

From such a study four groups may be made

- Group 1 showed an average Malignancy Index of 21.0
- Group 2 showed an average Malignancy Index of 23.11
- Group 3 showed an average Malignancy Index of 23.07
- Group 4 showed an average Malignancy Index of 22.81

The paper may thus be summarized

1 The cell types, the differentiation, and the anaplastic changes of carcinomas of the cervix have been studied. They were given a numerical value, the sum representing the histologic malignancy index.

2 Immaturity of the cells, a low degree of differentiation and a high degree of anaplastic changes are invariably associated with a high malignancy index.

3 The greater the maturity of the cells, the higher the differentiation and the less the anaplastic changes are, the lower will be the malignancy index.

4 The clinical malignancy of a carcinoma depends solely on the results of treatment obtained, provided the same method of treatment was used in each case. The extent of the carcinoma influences the outcome then only if it has thereby become a systemic or generalized disease (Group 4 cases). A carcinoma contained within a well defined area and having a low malignancy index offers every hope for a relatively good prognosis.

5 Comparing the histologic malignancy index with the clinical findings or grouping of the carcinomas and excepting the Group 4 cases, it is found that a definite relation between the two does not exist.

6 The relation of the cell type to the histologic malignancy index is definite. The unripe cell type is almost always associated with a high malignancy index.

7 The relation of cell type to the clinical result is not as definite as the relation of the malignancy index to the clinical result. The malignancy index shows a definite or proportionate relation to the result of treatment obtained.

8 Considering the relation of the malignancy index to the clinical result and excluding the Group 4 cases, we may conclude that the pathologist can give definite information as to the degree of malignancy from a histologic examination expressed in numbers of the malignancy index, which will enable the clinician to choose those cases of carcinomas which may respond with fair prospects to radiation treatment.

AGGLUTINATION TESTS A Simple Reaction for Detecting the Binding of Agglutinins by Difficultly Agglutinable Suspensions, Mudd, S Jour Immunol, 1927, vol. 2, 113

The resuspension reaction consists essentially in centrifuging the bacteria after exposure to serum and then resuspending the sediment by shaking. The intensity of the reaction is given by the size of the resulting clumps.

The bacterial suspension and serum dilutions are prepared, mixed, allowed to stand and the macroscopic agglutination readings are made in the usual way. All tubes are then centrifuged until clear, the salt solution controls are usually the last to clear and serve to indicate when centrifugation may be stopped. The tubes are then arranged serially in a rack with the controls and several of the highest serum dilutions in the middle. The rack is held with one hand at each end and shaken with an even backward and forward motion so as to make agitation of all tubes as nearly equal as possible. The control tubes are watched carefully during the agitation and as soon as their sediment has again become evenly suspended shaking is stopped. The tubes containing well sensitized bacteria still show flocculi increasing in size to the highest serum concentrations. Many of these clumps may break up if agitation is too prolonged, however, too low an apparent titer resulting.

After the results of the first resuspension are recorded it is often advantageous to make a check test. The tubes are accordingly again centrifuged until clear. The supernatant

fluid may be decanted by a quick inversion of the tubes without serious loss of sediment. Two drops of isotonic NaCl solution are then added to each tube. They are arranged in a rack and shaken as before but more gently until the control tubes become even. Flocculi are again recorded. The results of the two resuspension tests are usually in essential accord, and either one will often be sufficient by itself.

It is important that the internal diameter of all the test tubes used in this test, and the volume of liquid in each be approximately the same. A given amount of agitation will ordinarily break up the clumps less in narrow than in slightly wider tubes and less with large than with smaller volumes of liquid.

COLORIMETRY A Simple Colorimetric Method for the Determination of Iodine in the Urine Yoshimatsu S and Sakurada H Tohoku Jour Exper Med 1026 viii, 4, 107

The method depends upon the reduction of silver iodide previously dissolved in potassium cyanide solution and the production of a dark brown color which is applied to colorimetry. The reduction is effected by sodium sulphide.

Solutions required

1 Standard silver nitrate solution A Dissolve 1.338 gm of silver nitrate in distilled water and make up to 100 cc. 25 cc of this solution is made up to 500 cc with distilled water. 10 cc of this solution equals 0.001 gm of iodine.

2 Standard silver nitrate solution B .901 gm of silver nitrate is dissolved in distilled water and made up to 100 cc. 25 cc of this solution is made up to 500 cc with distilled water. 10 cc of this solution equals 0.001 gm of sodium chloride or 3.04 mg of chlorine.

3 Nitric acid (5N)

4 Concentrated silver nitrate solution Silver nitrate 50 gm aqua destillata ad 100 cc

5 Aqua ammoniac (10 per cent)

6 Potassium cyanide (5 per cent)

7 Gelatine solution (10 per cent) Ampoules for injection may be used with satisfaction

8 Fuming nitric acid

9 Chloroform

10 Potassium iodide solution (13 per cent)

11 Sodium sulphide (10 per cent)

METHOD

Determination of amount of urine to be used for analysis

Rinse 0.05 cc (0.5 mg of iodine) of 13 per cent potassium iodine solution into a 25 cc calibrated test tube. Put 0.5 cc of urine in another test tube of the same kind. Add to each tube 25 cc of distilled water and 3 drops of fuming nitric acid. Make up to about 10 cc with chloroform. Then mix and compare the colors. If the urine tube is much lighter add another 0.5 cc of urine again and again until an approximate intensity of color is reached. Then the whole amount used for the test is one tenth the amount to be used for the quantitative determination by the present method.

Method of analysis—Measure an optimum amount of urine for one sample as described above. Then pipette a certain amount (usually 1 cc is sufficient) from this measured quantity into a centrifuge tube of 25 cc contents (chloride tube) for the determination of chlorine. Divide the rest of the sample together with rinsings of the pipette (as little water as possible should be used for rinsing) in two or four centrifuge tubes of the same contents. Add to each tube the same amount of water and 1 cc of nitric acid (3) and then 2 cc of concentrated silver nitrate solution (4). Stir and centrifuge until the supernatant fluid becomes clear and remains so on a further addition of drops of silver nitrate solution. Pour off the supernatant fluid and wash the precipitates with distilled water three times. Add 1 cc of aqua ammoniac (5) to the chloride tube above mentioned and about half an amount

of the same corresponding to the urine quantity to all the other tubes. Stir well and centrifuge again. Transfer the supernatant fluid of the chloride tube into a 100 cc volumetric flask and make up to about 80 cc with distilled water. Put 10 cc of standard silver nitrate solution B (2) into another 100 cc volumetric flask and pour in 2 cc of aqua ammonia and 50 cc of water. Add 1 cc of gelatine solution (7) and 2 cc of sodium sulphide (11) to each flask. Wait one minute and fill up to mark with water. Compare the colors thus developed in a Duboseq colorimeter by setting the standard at 200 mm. The calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.005 \text{ gm} = \text{N}\text{r}\text{Cl or } 3.04 \text{ mg} = \text{Cl in amount of urine taken}$$

As to the other tubes (iodine tubes) pour off the supernatant fluid and wash the precipitates three times. Add 1 cc of potassium cyanide solution (6) to each tube and dissolve the precipitates. Transfer the contents of all the tubes into one and the same 50 cc volumetric flask. Put into another 50 cc volumetric flask exactly 10 cc of standard silver nitrate solution A (1) and add 1 cc of potassium cyanide solution (6). Then add 0.5 cc of gelatine solution (7) and 2 cc of sodium sulphide (11) to each flask. A brown color develops immediately. Wait one minute and fill each flask up to mark with water. Compare the colors in a Duboseq colorimeter, setting the standard at 200 mm. The calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.005 \text{ gm} = \text{I in amount of urine taken}$$

AGGLUTINATION A Rapid Method for the Macroscopic Agglutination Test, Noble, A Jour. Bacteriol., 1927, 14, 5, 287

The test is made in small test tubes (75 x 13 mm.)

Place 0.1 cc of heavy bacterial suspension in a tube and add 0.1 cc of the serum diluted 1:10 with N/S. In another tube (control) place 0.1 cc of bacterial suspension and 0.1 cc of N/S.

Place the tubes in a rack and, inclining the rack so that the mixture flows up the tube about one inch, shake for two minutes. Then add 0.8 cc N/S to each tube and read for agglutination.

The serum dilution cited is 1:100. Dilutions may be made in series as desired.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building
Richmond Va

*Neuberger's History of Medicine**

THIS section of Neuberger's monumental history deals with medicine in the Middle Ages. The entire work is not yet available in English but the publishers are to be congratulated on going ahead with the publication of that section which is now available.

So many of the medical histories pass over the Middle Ages with scant attention because so little of real progress was contributed in this Dark Age in medicine. It one will but stop to consider, however, one must realize that there must have been events during these times many of which are of decided historic interest, even though they do not mark steps of great progress in our knowledge of the arts and sciences.

The story of the school of Salerno in its prime and during its decadence is alone an hour of enjoyable reading. The Arabic influence upon western medicine known to all, is more readily understood after reading Neuberger. We usually understand that the writings of Hippocrates were preserved for us in the Arabic until the Renaissance. But when we understand how they were translated from the Arabic, we are in a better position to understand how fragmentary, incomplete and erroneous our present knowledge of the Hippocratic writings is. The Italian translator having little or no familiarity with Arabic, caused the text to be read to him in the Spanish vernacular by some one well acquainted with the language and wrote it down at once in Latin from this dictation. The slavish translation word for word makes it readily comprehensible that grotesque perversions of meaning are not uncommon. Frequently it appears as though the reader was left by the unintelligent translator to read his own meaning into the confused text.

We are inclined to think of the early Church as having stifled medicine. In a sense this is true, but at the same time it was the church and the church alone that in its monasteries maintained what few traditions were carried on through the Dark Ages.

Neuberger's volume accomplishes one feat which is too often lacking in medical histories, it gives the reader a sensation of actual personal acquaintanceship with the actors as they pass on and off the stage.

The Specialties in General Practice†

THIS work deals with those more common diseases which by reason of their location are usually treated by the specialists but which could probably be treated as satisfactorily by the family physician provided he knew the most acceptable treatment to be applied and were willing to apply it. Those diseases whose treatment requires special skill or the use of unusual instruments are not detailed.

One may pick up the volume in a search for information concerning the treatment of a special disease. But it is pleasant reading, and one will read on only to find later to his

History of Medicine. By Dr Max Neuberger, Professor of Medical History in The University of Vienna. Translated by Ernest Playfair, M.B., M.R.C.P. In Two Volumes. Vol. II Part I. Paper. Pp. 13.. Oxford University Press, American Branch, N. Y.

†The Specialties in General Practice. Compiled by Francis W. Palfrey, M.D., Instructor in Medicine at Harvard University in Collaboration with The Author's Name on Page 9 in Their Respective Subjects. Cloth. Pp. 748. W. B. Saunders Company, Philadelphia. Pa. 1927.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

surprise that he has quite unconsciously covered quite a large portion of the book and at the same time quite a wide range of medicine. One finds no revolutionary in the therapeutic outlines and gains the impression that the work is based on didactic lectures to senior medical students. But at the same time the reader is surprised to discover how refreshing it is to renew acquaintance with old friends and on every page or two pick up a valuable tip either new or forgotten which he will undoubtedly have an opportunity to apply in his work in the not far distant future.

We might characterize the volume briefly "An Excellent Post Graduate Course" for the general practitioner. Those diseases which rightly fall within the domain of internal medicine are not discussed within the volume. In those conditions which are discussed, diagnosis is touched upon but treatment in particular is emphasized.

*Physical Diagnosis of Diseases of the Chest**

HERE is a new method of attack and treatment. One hundred pages devoted to fundamental considerations, such as, methods of examination, the physics of sound, and the technic of examination. Twenty odd on the examination of the healthy chest. All of this before there appears any discussion of the lungs in disease, and this latter section is no longer than the first. To one who is impatient to get to the end of a book or to get down to the so called practical aspects this will be a bit tedious. But to one for whom a thorough grounding and understanding of his subject is the first consideration, the volume should be most alluring.

Dr Bushnell devotes considerable space to the tuberculous chest with the remark that after one has mastered the diagnosis of pulmonary tuberculosis in its various forms, the diagnosis of other diseases of the lungs is found to be relatively easy. His wide army experience with early and incipient tuberculosis makes Dr Bushnell an authority on the subject.

Dr Pratt's discussion of the diagnosis of diseases of the heart follows more closely the conventional lines, but his presentation is built upon a wide experience, and a clear insight which makes his work easy reading and should enable one to apply the principles enunciated with distinct success. The illustrations are abundant and excellent. Dr Pratt, like the late Sir James Mackenzie, emphasizes that the true cardiologist is the man, who when the occasion arises, can successfully dispense with the various instruments which have been devised for cardiac study.

Symptom Diagnosis†

THE author outlines three outstanding purposes of the volume, first, to aid the busy physician in the diagnosis of his case by allowing him to quickly reduce the number of possibilities to a small list, second, to prevent the oversight of important considerations, and third, to make the medical man more observant of the characteristics of the symptoms of disease. It is purely a reference manual and as such is excellently arranged. The examiner can find in a few minutes the various causes both common and uncommon of the patient's chief complaint. Bearing these in mind he may then execute his physical examination, searching for evidence of one or another of these causes. A definite tendency in the examination of the patients is to suspect a particular malady after having heard the patient's story and, therefore to carry out the examination with the perhaps unconscious intention of confirming this suspicion. This is probably one of the most frequent causes for error in diagnoses. Suspended judgment throughout the examination is essential but its value is surely enhanced if at the same time one is intelligently on the *qui vive* for all

*Physical Diagnosis of Diseases of the Chest. By Joseph H. Pratt, A.M., M.D., and George E. Bushnell, Ph.D., M.D. With 106 Illustrations. Cloth. Pp. 522. W. B. Saunders Company, Philadelphia, 1925.

†Symptom Diagnosis: Regional and General. By Wilfred M. Barton, A.M., M.D., F.A.C.P., and Wallace M. Yater, A.B., M.D. Cloth. Pp. 851. D. Appleton & Co., New York, 1927.

possibilities. For the purposes for which it was prepared this volume may be highly recommended. The outlines are brief, concise and to the point and with the help of the table of contents and index the different symptoms are found most readily.

The authors are to be felicitated on a happy selection in dedicating the volume to Dr George M. Kober, venerable dean among Washington's physicians, a man of delightful personality, a guiding spirit of Georgetown University.

*Diet and Dietetics**

A COMPILATION by various British authors, most of whose names are well known on this side of the Atlantic. As the editor remarks in his preface, until our knowledge of physiology is more perfect than at present, the scientific basis of dietetics must be an unstable one. Nevertheless, patients must be dieted and the physician must be guided by the teachings of history, by experimental physiology and by clinical experience in the proper regulation of the diet.

Sir Lauder Brunton opens the discussion with an introduction on the general principles of dietetics. Next follows a chapter by Harry Campbell on the evolution of man's diet in which he brings out the noteworthy fact that man has advanced to his present state on a diet which is in great part carnivorous. One may therefore rightly question the claims of the vegetarian that flesh is not appropriate to man.

Dr E. I. Spriggs describes the physiology of digestion, absorption and nutrition and devotes many pages to the diet in health. Edmund Cantley tells us that proprietary foods are not necessary either in sickness or in health. The greater the knowledge possessed by the individual of the composition of natural and proprietary foods and of the various means of modification by simple home methods, the less will he find it necessary to have recourse to the manufactured article and the better will be the results of his dietetic treatment. Although unnecessary, it must nevertheless be clearly realized that such foods are sometimes of greatest value. It is equally important to realize that a knowledge of the composition is essential to the proper utilization of any proprietary food. The composition of a great variety of proprietary foods is described in detail.

Dr Harry Campbell's chapter on alcohol is especially readable. Alcohol has been known to man probably for about thirty thousand years coming into existence with the development of agriculture. Only for fifteen thousand years has it been used more or less to excess because prior to that time there were no facilities for storage. Dr Campbell does not make out a good story for alcohol either in health or disease. Alcohol leads to the production of heat which however is met by a corresponding or even greater heat loss. It may furnish energy but it diminishes the capacity for sustained muscular work. It promotes the laying on of fat and diminishes the amount of food needed to sustain body weight. Even the moderate drinker has a poorer life expectancy than has the total abstainer.

Dr Campbell finds a better remedy than alcohol in nearly all of those diseases in which alcohol may be prescribed. Occasionally it may be of distinct value however in acute cardiac emergencies or in fatal diseases such as sarcoma or advanced phthisis.

The appropriate diets in various diseases are contributed by different authors. Many of the 'cures' used at health resorts on the continent and in England are described. This should give the volume value as a reference work in America.

The discussion of diabetes is too brief.

The presentation of diets for individual diseases follows the usual routine and is open to the usual criticism. Too much attention is paid to those diseases in which diet is of minor importance and too little to those fewer diseases in which diet is a major consideration. The section on diet in infancy and childhood is good. So also is the short chapter on diet in old age.

* A System of Diet and Dietetics. Edited by G. A. Sutherland, M.D., F.R.C.P. Second Edition. Cloth. Pp. 893. Physicians and Surgeons Book Company, New York.

*Histology of the Endocrine Organs**

TEXTBOOKS on histology describe in detail the microscopic picture of the various organs of internal secretion. But with few exceptions account is not taken of the age factor, and yet as one will see at a glance age may play a distinct part in altering the histologic picture of this type of tissue. Dr. Cooper has made careful comparative studies of the thyroid, parathyroid, pituitary, suprarenal, and thymus glands at different ages. All the work was with human material. The author observed decided differences in different age periods. She did not make a study of the sex glands.

Diseases of the Skin†

A RICHLY illustrated textbook on dermatology, of encyclopedic proportions. Most physicians and too many pseudodermatologists know far too little of the great variety of afflictions to which the skin is heir. So many cutaneous manifestations respond readily to a few drugs such as sulphur, mercury, zinc oxide, tar, or arsenic internally, that the usual procedure is, we venture to say, the numbing offhand of an indistinct or nondeterminative diagnosis and the prescribing of some old favorite ointment. The condition failing to respond, the physician scratching his head, makes some remark about an unusually refractive case, and the patient continues with his disease. In scarcely any domain of medicine is it so true in dermatology, that, the correct diagnosis having been arrived at, the indications are clear cut and are specific for each condition. And in diseases of the skin where all or nearly all of the pathologic alterations are so accessible for study, in justice to his own self-respect, the physician should not be content without the most thorough and painstaking diagnostic scrutiny.

A volume such as Sutton's in which the illustrations are so clear and abundant and in which the text is thoroughly descriptive will serve as an excellent reference manual.

To those working to keep abreast of the more recent therapeutic advancements in dermatology, the reviewer would recommend particularly the chapters in the Parasitic Skin Infections, especially the mycotic disorders.

Textbook of Pathology‡

A TEXTBOOK of general and special pathology reflecting particularly the concepts in pathology as developed in the University of Edinburgh. The text is clearly written, the headings and the points for emphasis are easily followed, being printed in bold face type and the work is most profusely illustrated with excellent photographs, colored plates, and photomicrographs.

The interest throughout the work is preeminently in structural pathology, there being practically no discussion of clinical pathology, although a brief chapter is devoted to immunity.

*The Histology of the More Important Human Endocrine Organs at Various Ages. By Eugenia R. A. Cooper, M.D., Demonstrator of Anatomy and Late Leech Fellow of the Victoria University of Manchester. Cloth. Pp. 119. Illustrated. Oxford University Press. American Branch, New York.

†Diseases of the Skin. By Richard L. Sutton, M.D., LL.D., F.R.S. (Edin.), Professor of Diseases of the Skin, University of Kansas School of Medicine, Assistant Surgeon, U. S. Navy, Retired, Member of the American Dermatological Association, Dermatologist to the Atchison, Topeka and Santa Fe Hospital Association, Dermatologist to the Christian Church Hospital. 1237 illustrations and 11 colored plates. Seventh Edition, Revised and Enlarged. Cloth. Pp. 1694. The C. V. Mosby Co., St. Louis, Mo.

‡A Textbook of Pathology, General and Special, for the use of Students and Practitioners. By J. Martin Beattie, M.A. (NZ), M.D. (Edin.), M.R.C.S., L.R.C.P. (Lond.), and W. E. Carnegie Dickson, M.D., B.Sc., F.R.C.P. (Edin.). Cloth. Illustrated. Pp. 1103. C. V. Mosby, St. Louis, 1926.

*Electrothermic Methods in Neoplastic Diseases**

A HANDBOOK on diathermy electrodesiccation and electrocoagulation. The author presents a brief description of electricity, types of electrical current, chemical and physical effects of these currents, their use in medicine and surgery, and the instruments best suited for this manipulation.

A large portion of the book is devoted to the action of electrodesiccation and electrocoagulation in specified conditions. The author does not limit himself to neoplastic diseases but describes the results of this treatment also in such conditions as fissure, fistula, hemorrhoids, corneal ulcer, chancreoid, x-ray dermatitis, tattoo marks, tonsil removal, and chronic ulcers.

The last chapter consists of a series of practical laboratory exercises in the use of the machines using the operator himself, meat, potatoes, soap, etc., as subjects.

The Abdomen in Labor†

THE author takes exception to the custom, apparently altogether too prevalent in England, of making routine vaginal examinations of the parturient woman. The obstetrician is inclined to concentrate his attention on the uterine cervix, a part which he cannot see, and with his eyes at his finger tips attempts to gain the information he thinks he requires from the insignificant fraction of the uterus projecting into the vagina. But the cervix and os uteri play not an active part but a passive part in labor. The changes forced upon the inert cervix are entirely the result of the action of the great body of muscle above it. It is not in the cervix but in the powerful contracting muscular mass of the uterus that the essential phenomena of labor occur.

The author concludes after years of study of the abdomen during labor that a wealth of valuable and instructive information is usually lost to the obstetrician because of his failure to inspect the abdomen and to gain that knowledge which is made available to palpation. By inspection of the abdomen he implies, of course, a really intelligent study of the contours and prominences at various stages, particularly when the patient is in pain and not in pain. Dr. Porritt feels that as a rule the position and presentation and the presence of most abnormal conditions, except those directly at the cervix, can be as readily identified by abdominal examination as by vaginal examination and with far less risk to the patient.

His description of the findings within the abdomen during labor is illustrated with drawings made at the bedside.

Old and New Viewpoints in Psychology‡

AN AUTHORITATIVE critical discussion of certain phases of psychology which have caught the popular fancy and have been adopted by pseudoscientists usually for personal gain. This includes particularly Freudian psychoanalysis, intelligence tests, spiritualism, and character analysis. There is also a delightful chapter on the psychology of the comic.

The author has no objection to intelligent tests as one form of mental measurement when properly applied. The trouble is that in the past it has been too frequently misapplied. The Binet-Simon tests were developed as a means of measuring the intelligence of children, and their evolution covered a number of years in which painstaking study was made. At the onset of the war an attempt was made to develop similar tests for

Electrothermic Methods in Neoplastic Diseases. By J. Douglas Morgan, B.A., M.D. *Electrothermic Methods or (Desiccation and Coagulation in the Treatment of Neoplastic Diseases. Designed as a Practical Handbook of Surgical Electrotherapy for the use of Practitioners and Students.* By J. Douglas Morgan, B.A., M.D. Cloth. Illustrated. Pp. 17. 1s. A. Davis Company, Philadelphia.

The Abdomen in Labor. Being a General Practitioner's Clinical Study of the Parturient Abdomen. By Norman Porritt, M.R.C.S., L.R.C.P. (Lond.), Consulting Surgeon, Huddersfield Royal Infirmary. Cloth. Pp. 76. New York: Oxford University Press, 1926.

Old and New Viewpoints in Psychology. By Knight Dunlap, Professor of Experimental Psychology in the Johns Hopkins University. Cloth. Pp. 166. The C. V. Mosby Company, St. Louis, Mo. 30.

recruits, many of which were unscientific, and therefore, unsuccessful. Thus the testing of an aviator for his reaction to various stimuli told nothing. But the more recent tests which measured his ability to manage a complicated series of reactions analogous to the operations and discriminations required of him in his airplane told a great deal.

The Binet-Simon test is not simply a language test. A moron may have considerable fluency in language, but he does not pass the Binet-Simon test. This test measures acquisition almost exclusively. From the measure of what knowledge the individual has actually acquired we estimate his future capacity to acquire. The individual is a moving body and by plotting the course he has passed over we predict future developments.

The application of even the best intelligent tests requires intelligence. A school teacher or a nurse or even a physician cannot read over the principles of the test and then apply them correctly. The matter of interpretation requires experience. Too frequently inexperienced testers have rated feeble-minded children as normal and normal or even exceptionally bright children as feeble-minded.

The statement has been made that the average mental age of the draft was 13.5 years. This is viciously misleading, for the Binet-Simon scale of ages stops at fourteen, the following two grades being adult and superior adult. These latter are sometimes specified as sixteen and eighteen years respectively. It is readily seen, therefore, that an averaging of mental ages, not taking this into account, will explain the erroneous impression given.

Dr Dunlap believes in intelligence tests properly applied. This means a special set of tests for each individual problem. One set for switchboard operators, another for telegraphers, another for jobs in rubber factories, etc.

The author has scant patience for the Freudian psychology and psychoanalysis which he discusses at some length. There is no basis in experimental psychology for the so-called subconscious mind. "Nothing could be more vicious or absurd than the doctrine of repression. Actual repression is the only salvation of man if civilization is to continue, and the ability to repress effectively is the greatest asset a human individual can have." "Nothing is more weakening than to keep thinking of past mistakes and illicit desires. The adolescent boy and girl need to have their attention drawn away from the surging desire of sex and turned in other directions. Freud and his disciples have contributed nothing of value to psychology, and if they have contributed anything to medicine, it is rather discreditable to medicine to have been so far behind the progress of psychology that it could profit by this mixture of psychology and superstition."

Dunlap who has investigated spiritualism in detail finds not a single case in which the claims can be proved or are free from flaws. His discussion is rather of the mental make-up of those who interest themselves in this pastime.

So-called character analysis has developed from the old phrenology, has no scientific basis whatever, and is practically without exception exploited for commercial purposes.

In the psychology of the comic, Dr Dunlap classifies those things that we term "funny" in various grades from the lowest, the pleasure and amusement that the brute and the savage take in torture, up through horseplay, practical joking, puns, and so on to the idle wittiness. After telling us why we consider these things funny he then offers an explanation as to why we laugh.

*Thomas Sydenham**

SYDENHAM like his follower Boerhaave and like Sir James Mackenzie, our modern Sydenham, had little patience with experimental medicine in so far as it had developed during his day, but possessed a clinical acumen that has scarcely been equalled by any other physician and has been excelled only by Hippocrates himself. Dr Riesman's

*The Sydenham Clinician. By David Riesman, M.D. Professor of Clinical Medicine, University of Pennsylvania. 46 pages. Paul B. Hoeber, Inc. N. Y. 1926.

presentation of this remarkable man gives us the picture of a very human individual dictatorial impatient and yet conscious of his own mistakes and shortcomings. The small volume provides a most enjoyable half hour.

Examination of the Patient and Symptomatic Diagnosis†

IN ATTEMPTING to arrive at a diagnosis of a patient's illness the more thorough the history obtained the greater will be the number of symptoms both past and present which will be utilisable in reaching a conclusion. The symptoms having been discovered turn now to a standard work on medicine and you will find that diseases not symptoms form the basis of classification. It may then be necessary to search through the description of many diseases before discovering that into which the particular symptomatology under consideration fits completely. Dr Murray has in his volume roughly reversed this procedure in that he takes up subjective and objective symptoms and under each symptom heading he enumerates the possible etiologic factors pointing out at the same time what additional or confirmatory questions should be asked or examinations made in order to reach a correct conclusion.

The Peaks of Medical History‡

ONE cannot obtain too extensive a historical background for the subject which is one a life work. Dr Dana touches only the high spots but in doing so he marshals a galaxy of names all of which should be familiar to every student of medicine. In a book the size of this much of interest must be omitted and in perusing the very readable pages one has the feeling that he is witnessing a rapidly moving panorama and regrets his inability to stop here and there and develop a more personal acquaintance with many of the actors as they slip by.

The work might be described as a beginner's history. As such it whets the appetite for more. This was obviously the author's intention for in an appendix entitled Bibliographical Notes he directs the reader to those works carrying greater detail.

But even one who has a fairly general knowledge of medical history will enjoy reading Dana's volume for no two such works are entirely alike and here and there in the pages one picks up curious and interesting bits of new information.

The illustrations are excellent and numerous. In addition to portraits of the great leaders and thinkers there are many which indicate medical activities in the different epochs which as the author states are kinetic rather than static in their character.

The craftsmanship of the book as is usually the case with this publisher is excellent.

Examination of the Patient and Symptomatic Diagnosis. By John Watts Murray, M.D. 16 Illustrations. Cloth. Pp. 841. C. V. Mosby Company, St. Louis, Mo. 1926.

†*The Peaks of Medical History*. (An Outline of the Evolution of Medicine for the use of Medical Students and Practitioners). By Charles L. Dana, A.M., M.D., LL.D., Professor of Nervous Diseases, Cornell University Medical School, Late President of the N. Y. Academy of Medicine. Illustrated with 40 full page plates and 16 text illustrations. Cloth. Pp. 105. Paul B. Hoeber, Inc., New York. 1926.

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EDITORIALS

Recent Studies of Measles

THE first mention of measles as a distinct disease is found in the writings of Ahiun, a physician of Alexandria in 610 A D, although it was not adequately described until the time of Rhazes, 900 A D, who distinguished it from smallpox. It remained for Sydenham, in 1655, to separate measles from scarlet fever and to draw attention to it as one of the most important of "the diseases of childhood."

Because of its frequency, its importance, and the often serious nature of its sequelae, a great deal of study has been devoted to the evolution of methods for its prophylaxis and control, and until these are satisfactorily achieved, it is of interest to review from time to time the results of studies applicable to the problem.

The etiology of measles is still under discussion.

The diplococcus described by Tummehff and her coworkers has been the subject of much study, as a result of which a skin test for susceptibility to measles has been devised^{1 2} and other evidence adduced in support of their contention as to its etiologic relationship.

Cary and Day³ report the isolation of a similar organism from 98 per cent of throat cultures, 50 per cent of conjunctival cultures, and 33 per cent of blood cultures in early measles with which they were able to produce rashes and febrile reactions in rabbits, while Ferry and Fisher⁴ report the preparation of a measles toxin from filtrates of the aerobic, green producing streptococcus isolated by Ferry from the blood in early measles.

Hibbard and Duval⁵ are convinced that the causal agent of measles exists in the circulating blood during the febrile stage and that it may be propagated *in vitro*. While they encountered an organism similar to that described by Tunnell⁶, they are noncommittal as to its etiologic relationship to the disease, which indeed, is so far the status of all the organisms as yet isolated in this disease. In a continuation of their work the same authors elicited further evidence suggesting a definite relation of the Tunnell diplococcus and measles,⁶ although holding a final conclusion *sub judice* until further investigations shall determine whether an organism or as suggested by Park⁷ and Degwitz,⁸ a filtrable virus.

That the problem is one well meriting study is evidenced by the regularity with which the disease appears and the high incidence of susceptibility, a recent survey showing that approximately 90 per cent of males and 95 per cent of females over twenty have had measles.⁹

In the absence of specific information as to the cause and mechanism of the spread of measles, it is more or less generally accepted that, for the present, attempts at its complete eradication are foredoomed to failure and for this reason competent observers^{10, 11} suggest a concentration of effort to protect especially children under three years of age, as fully 70 per cent of all measles deaths fall within that age period.

That such a plan is practical demands no new methods but merely an intensified application of known procedures and will produce results, as proved by the report of its practical trial in an epidemic at Syracuse during 1926-27 by Ruhland and Silverman.¹²

It is obvious that in the spread of communicable and infectious diseases the unrecognized case is a factor of great importance.

In the interest of early diagnosis Stimson¹³ in a timely and pertinent paper, comments upon the fact that it is not generally recognized that measles may often be predicted with some confidence before the appearance of Koplik spots and that it is highly contagious from one to two days before these pathognomonic signs appear.

He discusses in extenso the symptoms of measles, especially those of the stage of invasion and incubation, listing them in the order of appearance as follows:

- 1 Malaise, with which there may be headache

- 2 Fever

- 3 Eye signs (twelve hours later) puffiness of the lower lid, perhaps the measles line and the first sign of exanthem on the faces. In examining a patient with a cold therefore, one should examine habitually inside the lower eyelids and inside the cheek.

4 Twelve hours later come the three C's cough, coryza, and conjunctivitis

5 Twelve hours later appear Koplik's spots

6 Twelve hours later the rash appears

As Stimson says, the physician must be ever on the alert to make the earliest possible diagnosis, to *individually* isolate the patient on the first suspicion, and to protect him from secondary infection

Only by these means can the incidence, mortality, and sequelae of this very serious disease be at all controlled until more specific procedures are evolved

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—R A K

Skin Sensitivity and the Dick Test in Newborn Infants

THE introduction of the Dick test as a means of detecting those susceptible to scarlet fever has very naturally led to its extensive use in children

The similarity of the procedure to that used in the study of those susceptible to diphtheria, the fact that scarlatina convalescents not only give a negative Dick test but their serum when added to the toxin neutralizes its action in the skin of a susceptible individual, all justify the common assumption that a negative Dick test indicates a true antitoxic immunity

Observations by von Groer and Kassowitz¹ upon the immunity to diphtheria in very young infants as indicated by the Schick test and by the demonstration of antitoxin, showed, however, that while the skin of newborn infants, whose blood did not contain antitoxin, reacts to the Schick test in only about one-third of the cases tested (143), this reactive ability was acquired by the skin in all cases by three months of age

A similar investigation, but concerned with skin sensitivity to the Dick toxin, is reported by Cooke²—apparently the first work of this nature to be recorded

The investigation covers a study of 200 mothers and their infants during the first ten days of life and again from six weeks to three months later

In the entire group of infants 86 per cent were negative to the largest amount of toxin used (50 STD) and only 1 per cent was positive to the smallest dose (2 STD) When compared with the reactions of the mothers it is seen that

A All infants reacting in any degree had mothers whose skins were extremely or moderately sensitive

B More than 75 per cent of the infants with reactions had mothers whose skins were very sensitive

C When the mothers' tests were negative or only slightly positive, the infants' tests were always negative

It is of immediate practical importance to determine whether this negative reaction thus commonly encountered in the newborn is due to a lack of skin sensitivity or to the presence of antitoxin presumably transmitted from mother to infant Cooke's study was extended to include such a determination

A sufficiently extensive series of mothers' serum was examined to corroborate the conclusions of others that in those reacting to 2 STD toxin, antitoxin was present Whenever antitoxin was present in the infant's blood, Cook also found it in the mother's but the reverse was not true so that, at birth the presence or absence of antitoxin appears to have no relation to the presence or absence of skin reactivity to toxin, since many infants without antitoxin were negative to 50 STD of toxin When a number of the same infants whose skin sensitivity had been tested at birth were again tested from six weeks to three months later there was some skin sensitivity to toxin in about one third but only a few were sensitive to small amounts, this development of toxin sensitivity occurring mostly in infants of very sensitive mothers

From these studies Cooke concludes that the skin in the newborn is not usually sensitive to large doses of scarlatinal toxin that this lack of sensitivity is not due to the presence of antitoxin, and that skin sensitivity to toxin may develop after some weeks, most often during the first months of life, in a larger proportion of infants who had no antitoxin at birth than in those whose blood contains antitoxin

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—R A K

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THE SEVENTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

THIS meeting surpassed all the previous ones both in the scientific character of the papers presented, which have always been good, as well as in the opportunities afforded for intimate personal chats among members for the discussion of problems relating to the practice of clinical pathology. Thanks to the excellent arrangements of the local committee at Minneapolis headed by Dr Charles R Drake with the valuable assistance of Dr G L Meikert, practically all of the delegates were housed in the same hostelry, the Leamington Hotel. The scientific and commercial exhibits proved highly interesting due to the untiring efforts of Dr A C Bowers and Dr Kano Ikeda. There were a good many present who had never missed a meeting and others who had come for the first time. A wonderful family spirit prevailed, no distinction was made between the younger men who had just joined and the old war horses who were veterans in the movement. Everybody was made to feel at home and companionship reigned.

At each noon and evening meal there was a spontaneous gathering of Fellows at a large round table in the dining room where informal discussions were held on a great variety of subjects pertaining to clinical pathology, both scientific and economic. This unofficial feature formed a delightful part of

the program of our convention and helped to cement the fast friendships that have been formed among our members since the inception of our organization. Practically all the Fellows promised to make all endeavors to see each other again at the next convention, so that they may carry away with them for the rest of the year the stimulus of meeting their fellow workers and the pleasant experiences of their intellectual encounters with their colleagues in the profession.

In another column will be found the minutes of the convention which will give those who were unable to be present, as well as the many friends of the Society who are interested in its welfare an idea of the program of the meeting, as well as the newly added round table feature to which a pleasant evening full of delightful encounters and enlightening information, was devoted.

FRANK WILBUR HARTMAN

Detroit Michigan

President, 1928-1929

Frank Wilbur Hartman was born in 1890. He obtained an A.B. degree from Knox College in 1913, an M.D. degree from the Medical Department of Johns Hopkins University in 1917. For two and one half years during the



war he was attached to the U. S. Naval Medical School and U. S. Naval Hospital, Washington, D. C. as pathologist and instructor in pathology. Dr. Hartman was a Lieutenant in the Medical Corps of the U. S. Navy. During this time he also had an assistantship at the George Washington University, Washington, D. C.

Following the war Dr. Hartman was for nearly three years Pathologist and Director of Laboratories at the Scott and White Clinic and Hospital and Santa Fe Hospital in Temple, Texas.

Since August 1922, Dr. Hartman has been Pathologist and Director of Laboratories at the Henry Ford Hospital in Detroit. He is a

Association of Pathologists and Bacteriologists and American Society of Experimental Pathologists, also a member of the Bone Tumor Committee of the American College of Surgeons at the present time

Chief interests Relation of phosphorus and sugar metabolism
 Production and study of experimental nephritis
 Primary heart lesions produced by deep x-ray

JAMES HARVEY BLACK

Dallas, Texas

President-Elect

James Harvey Black, M.D., born in Huntington, West Virginia, March 27, 1884, is the son of a Methodist minister. Moved to Texas at age of twelve years. He received his academic education at Paris High School, Paris, Texas,



and Southwestern University, Georgetown, Texas, graduated in Medicine in 1907 from the Medical Department of Southwestern University. Was successively interne at St. Paul's Sanitarium, 1906-7, Instructor in Bacteriology and Histology, Southwestern University, 1907-8, Professor of Physiology and Bacteriology, id, 1912-15, Dean Southern Methodist University School of Medicine, 1914-15, Professor of Bacteriology and Preventive Medicine, Baylor University, 1915-17 and 1919-20, Professor of Pathology and Bacteriology, id, 1917-19, Professor of Preventive Medicine, id, since 1920.

Dr. Black is a member of the academic fraternity Kappa Alpha and the medical fraternity Theta Kappa Psi. He is a member of the Methodist Church and of the Masonic Fraternity, Dallas Country Club, Dallas University Club, and Town and Gown Club of Dallas. His scientific affiliations are County, State and American Medical Associations, American Society of Bacteriologists, American Microscopical Society, American Association for the Advancement of Science, American Public Health Association.

His chief interest is in Allergy and Immunology.

MINUTES OF THE SEVENTH ANNUAL CONVENTION OF THE
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS,
MINNEAPOLIS, MINNESOTA

The proceedings were held in the Leamington Hotel, Minneapolis Minnesota, June 8, 9, and 11 1928

The meeting was called to order Friday, June 8, 1928, at 9 A M by the President, Dr A H Sanford A short business session was held in which the first order of business was the appointment of a Committee on Necrology, as follows Dr Philip Hillkowitz Denver Colorado, *Chairman*, Dr Frank W Hartman Detroit, Michigan Dr Robert A Keilty Washington, D C and Dr Chas C W Judd Washington D C and a Nominating Committee consisting of Dr A H Schade Toledo Ohio *Chairman* Dr C W Maynard Pueblo Colorado and Dr Nathan Rosenthal New York City

Dr Charles R Drake Chairman on the Committee on Local Arrangements, made several announcements

Dr Philip Hillkowitz Denver Colorado proposed an amendment to the By Laws to read as follows Article III Section 2 The annual dues for active and associate members shall be Ten Dollars (\$10 00), payable before or on the date of the annual meeting

There being no further business the regular scientific session of the Society presented the following program

The Interpretation of the Wasserman Test By B Markowitz, M D Chicago, Illinois
Complement Preservative—A Practical Study By B W Rhamy, M D, Fort Wayne, Indiana

Both papers were discussed by Dr H C Sweeney Chicago Dr J J Moore Chicago Dr Wm G Exton Newark N J, Dr F E Sondern New York Dr A H Schade, Toledo Ohio, and closed by Dr B Markowitz and Dr B W Rhamy

The Vegetative Nervous System in Epilepsy By A M P Saunders M.D, Chicago, Illinois (No discussion)

Method for Measuring the Bactericidal Action of Whole Blood Against Gram Positive Cocci By William Thalheimer M D, and Charlotte Colwell, A B Milwaukee Wisconsin (Discussed by Dr Robert F Maul Denver, Colorado Discussion closed by Dr Wm Thalheimer)

The Interpretation of Borderline Allergic Reactions By Warren T Vaughan M.D, Richmond Va (Discussed by Dr J H Black, Dallas Texas Dr Wm G Exton, Newark and Dr A H Sanford Rochester Minn The discussion was closed by Dr Warren T Vaughan)

Spectrophotometric Analysis of Blood Serum in Normal and Pathologic Conditions Study II By Charles Sheard Ph D T B Magath M D, and A E Osterberg M D Rochester, Minnesota (Discussed by Dr F E Sondern, and Dr Wm G Exton Discussion closed by Dr Charles Sheard)

Session adjourned

FRIDAY AFTERNOON, JUNE 8, 1928, 2 P M

The meeting was called to order by President A H Sanford and the scientific program continued

Association of Pathologists and Bacteriologists and American Society of Experimental Pathologists, also a member of the Bone Tumor Committee of the American College of Surgeons at the present time

Chief interests Relation of phosphorus and sugar metabolism
 Production and study of experimental nephritis
 Primary heart lesions produced by deep x-ray

JAMES HARVEY BLACK

Dallas, Texas

President-Elect

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and Southwestern University, Georgetown, Texas, graduated in Medicine in 1907 from the Medical Department of Southwestern University. Was successively interne at St Paul's Sanitarium, 1906-7, Instructor in Bacteriology and Histology, Southwestern University, 1907-8, Professor of Physiology and Bacteriology, id, 1912-15, Dean Southern Methodist University School of Medicine, 1914-15, Professor of Bacteriology and Preventive Medicine, Baylor University, 1915-17 and 1919-20, Professor of Pathology and Bacteriology, id, 1917-19, Professor of Preventive Medicine, id, since 1920.

Dr Black is a member of the academic fraternity Kappa Alpha and the medical fraternity Theta Kappa Psi. He is a member of the Methodist Church and of the Masonic Fraternity, Dallas Country Club, Dallas University Club, and Town and Gown Club of Dallas. His scientific affiliations are County, State and American Medical Associations, American Society of Bacteriologists, American Microscopical Society, American Association for the Advancement of Science, American Public Health Association.

His chief interest is in Allergy and Immunology.

- Retention of Urinary Constituents after Anastomosis of Urinary Bladder and Intestines
By F W Hartman M D Detroit, Michigan (Discussed by Dr F E Sondern
and closed by Dr F W Hartman)
- The General Practitioner and the Early Diagnosis of Cancer By Wm Carpenter Mac
Carty M D Rochester Minnesota (Discussed by Dr E L Miloslavich Dr J
J Moore Dr John W Gray Dr Robert A Keilty Dr Walter Freeman Dr A
S Giordano Dr A H Schade Dr B W Rhams Dr R E Myers Oklahoma
City Okla, Dr F H Lamb Davenport Iowa, Dr T C Terry Rochester Minn-
nesota and closed by Dr Wm Carpenter MacCarty)
- Rabies in Animals Demonstrations with Lantern Slides and Motion Picture Films By
E R Mugrage M D Denver Colorado (Discussed by Dr A H Sanford and
Dr W G Gamble Discussion closed by Dr E R Mugrage)
- The Sedimentation Time of the Blood in Jaundice By Nathan Roseenthal M D, M I
Blowstein M D and M Rachmulewitz M D, New York City (No discussion)
- Primary Carcinoma of the Fallopian Tube By Earnest Scott, M D and Margaret Oliver
Columbus Ohio (Discussed by Dr A S Giordano, and Dr C I Owen Closed
by Dr Ernest Scott)
- A New Method of Colorimetry By Wm G Extor M D, Newark N J (Discussed by
Dr Wm Thalheimer closed by Dr Wm G Extor)
- The Chemistry and Cytology of Serous Fluids By Alvin G Foord M D Cuy Young
berg Ph D and Vera Wetmore BS Buffalo, N Y (Discussed by Dr F E
Sondern Dr Kano Ikeda St Paul Minn and closed by Dr Alvin G Foord)
- Value of Nuclear Deviation (Schilling Classification) in Blood Examinations for Clinical
Medicine By F W Niehaus M D Omaha Nebraska (Discussed by Dr John
W Gray and Dr E R Mugrage Closed by Dr F W Niehaus)

Session adjourned

SATURDAY AFTERNOON, JUNE 9, 1924 2 P M

- The meeting was called to order by President A H Sanford and the scientific program
continued
- A New Method for Hemoglobin Determinations By Charles Sheard Ph D and A H
Sanford M D Rochester Minnesota
- Pertinent Facts Concerning Hemoglobin By C E Roderick M D Battle Creek, Michigan
Both papers were discussed by Dr Wm G Extor Dr A H Sanford Dr F E
Sondern, Dr A G Foord and closed by Dr Charles Sheard
- The Specificity of Bacteria to the Bacteriolytic Action of Chemicals with a Note on this
Application to Chemotherapy By Robert A Keilty, M D Washington D C
(Discussed by Dr Wm Thalheimer Dr C I Owen Dr F W Hartman and closed
by Dr Robert A Keilty)
- Results in Various Diseases from the Elimination of Foci of Infection and the Use of
Vaccine Prepared from Streptococci Having Elective Localizing Power By E
C Rosenow, M D, and A C Nickel M D Rochester Minnesota (Discussed by
Dr Robert A Keilty Dr F A Hecker, and closed by Dr F C Roebson)
- Further Studies on Brucella Abortus in Man By A S Giordano, M D and Marjorie
Ableson, South Bend Indiana (No discussion)
- The Etiology of Acute Leukemia By A S Rubnitz M D Omaha Nebraska (No dis-
cussion)
- Examination of Blood for Malaria By Leon S Lippincott M D, Vicksburg Missis-
sippi (No discussion)
- Purpura Hemorrhagica with Report of Three Cases By Oscar B Hunter M D Wash-
ington, D C (Discussed by Dr John W Gray)
- Estimating the Lacerment in Bactericidal Power of Individuals' Blood Produced by In-
travenous Injection of Typhoid Vaccine By Charlotte Colwell A B and J L
Lates, M D (by invitation), Milwaukee, Wisconsin Read by title

SATURDAY EVENING, JUNE 9, 1928, 7 P M, THE ANNUAL BANQUET

The Annual Banquet was held in the ballroom of the Leamington Hotel The speakers of the evening were as follows

Presidential Address By A H Sanford, M D, Rochester, Minn

The Cults By Wm O'Brien, M D, Minneapolis, Minnesota

Greetings from the American College of Surgeons By M T MacEachern, M D, Chicago, Illinois

Greetings from the American Medical Association By N P Colwell, M D, Chicago, Illinois

MONDAY MORNING, JUNE 11, 1928, 9 A M, THE BUSINESS SESSION

The business session was called to order by President A H Sanford

The reading of the minutes of the previous meeting was dispensed with as they had previously been published

The Report of the Executive Committee was presented by its Chairman, Dr John A Kolmer, Philadelphia The first matter presented was the question of charging a fee for the registration of technicians, which upon recommendation of the committee was to be deferred until after the report of the Committee on the Registration of Technicians

The next part of the report was the question of the official organ of the Society Dr Kolmer presented tentative articles of agreement with the C V Mosby Company, publishers of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, which the Committee had considered and recommended for adoption by the Society for a two year trial

The Committee reported that it was decided that the State Laboratory Problem would be submitted with recommendations through the Committee on Public Relations

The next section of the report of the Executive Committee was the matter of interpreting and amending the By Laws, if necessary, in relation to Associate Membership in the Society The Committee reported that in its opinion Section 3 of Article III of the Constitution is satisfactory but recommended special rigorous investigation of each applicant by the Board of Censors, requiring them to accept Article VIII, the Code of Ethics of the By Laws with the addition of a new section, 4, recommended to the Society for adoption as follows It shall be deemed unethical for an Associate Member of the Society to assume the independent direction of a laboratory of clinical pathology A motion was made and carried to accept this recommendation with the addition that Associate Members shall have no vote

Dr Kolmer stated that the Executive Committee had examined the books of the Secretary Treasurer and found them correct

The entire report of the Executive Committee was accepted by the Society

The Report of the Committee on Public Relations was given by Dr F E Sondern, New York City Regarding the State Laboratory question the Committee recommended that a similar committee be continued to make further effort with officials of the American Medical Association, the American College of Surgeons and the American Public Health Association to consider ways and means to correct existing evils through Commissioners of Health, Boards of Supervisors and others who determine the policies of the laboratories under their control

Relative to the Approval of Laboratories by the American Medical Association, the Committee recommended an endorsement of their efforts in the interest of improving the efficiency of private laboratories Report accepted A motion was made and carried that copies of the above resolution be forwarded to the Council on Medical Education and Hospitals of the American Medical Association

A motion was made and carried that the Committee on Public Relations be instructed to draw up a resolution on behalf of this Society which might go to representa

tives in different states, that they might use as an argument in favor of presenting this matter to state and county societies that the matter relative to the resolution be safeguarded by having a referendum adoption by the Executive Committee and then authorization that the Secretary's Office could send copies of this resolution to where it might do some good

The Report of the Committee on Exhibits was made by Dr Kano Ikeda St Paul, Minnesota, for Dr A C Broders Chairman, Rochester Minnesota The report was accepted by the Society A motion was made and carried that Exhibits, both scientific and commercial be handled solely by the Committee on Exhibits of this Society each year

Dr John A Kolmer Chairman of the Publication Committee reported that that Committee recommends the publication of a book of Approved Clinical Laboratory Methods under the auspices of this Society under the editorship of John A Kolmer with the assistance and collaboration of a Committee of the Society Report accepted

The Report of the Research Committee was presented by Dr H J Corper Chairman, Denver Colorado The Committee recommended the establishment of a yearly Ward Burdick Research Award Report accepted

The Report of the Committee on the Registration of Technicians was given by its Chairman, Dr Kano Ikeda The report incorporated the establishment of a Registration Bureau for Technicians and was accepted by the Society

Dr Philip Hillkowitz Chairman presented the report of the Committee on Necrology, telling of the deaths of six members of the Society in the past year, recommending that suitable resolutions of condolence be sent to their respective families They are Lieut Colonel H J Nichols Panama, Dr Dean N Bencom Denver Colorado, Dr Reed Rockwood Baltimore, Maryland, Dr James C Todd, Boulder Colorado Dr Ward Burdick Denver, Colorado and Dr Charles E Simon of Baltimore Maryland Report accepted

The Report of the Committee on Blood Groups was given by its Chairman, Dr F W Hartman Detroit recommending the adoption as a Society of the modified Landsteiner classification Report accepted

The Report of the Service Bureau Committee was read by Dr Henry C Sweany of Chicago, a member of the Committee in the absence of the Chairman Dr Robert A Kilduffe Atlantic City N J Report accepted

Dr George Ives Chairman of the Board of Censors, presented the report of the Board The following were elected to active membership Dr H E Butka Los Angeles Calif Dr Ralph A Fisher Enston Pa, Dr I A Nelson Tulsa Oklahoma Dr Roy W Hammack Los Angeles Dr John C Simpson Norristown Pa Dr Bowman C Crowell, Chicago Dr Wm McK Higgins New York City, Dr J Henry Litterer Nashville Tenn Dr Bust Litterer Miami Florida Dr Oliver S Hillman New York City Dr Victor Cefalu Seattle Wash Dr Marvin D Bell Dallas Texas Dr Kenneth Fowler Danville Pa Dr Louis P Hasting Burlington Vt, Dr S Lloyd Johnson Cantonville Md Dr Mary E Roche Norfolk Va Dr J H Robinson Temple Texas Dr Wm F Jacobs Buffalo N Y Dr P C Carson Denver Dr C J Bueher Philadelphia Dr Allen C Nickel Rochester Minnesota Dr Thomas Capgan Washington D C Dr Walter M Simpson Dayton Ohio Dr Sidney C Dalrymple Newton Lower Falls Ma Dr Carl H Reuter Springfield Ohio Dr F C Rosenow Rochester Minn Dr Wm Thalheimer Milwaukee Wis Dr Benjamin S Khuri Cleveland Ohio Dr George T Caldwell Dallas and Dr Fred H Stangl St Cloud Minnesota

To associate membership Dr Fred Boerner Dravel Hill Pa Dr Israel Davidsohn Philadelphia and Dr Clarence Z Carber Detroit Mich

Meeting adjourned at 1 P M

historic interest. Therefore, only such studies as are pertinent are included, these for the most part having been published in the light of modern advances in the knowledge of hydrogen ions. No attempt has been made to present references in their chronologic order. But so far as possible, some semblance of anatomic order has been observed, beginning with the duodenum.

For many years it was taught that neutralization of the acid chyme took place within a few inches distal to the pylorus, and that the intestinal reaction remained neutral or nearly so thereafter. There is at present, however, considerable experimental evidence to indicate that this is not always the case, that the duodenum may remain acid throughout its entire length. Grayzel and Miller⁵ made hydrogen-ion determinations upon the duodena of thirty-two dogs. These animals were fed on a variety of diets. The nearest approach to alkalinity was found in six dogs on a rachitic diet. The average duodenal P_H of these animals was 6.59. The average of ten dogs on a normal diet was P_H 5.91. The determinations were made both electrometrically and colorimetrically. Long and Fenger⁶ studied the reaction of intestinal contents of men. One subject was given an abundant diet of toast, figs, milk, and coffee. The Rehfuess tube was taken soon after breakfast, and allowed to work down through the pylorus. An hour after this the tube was about six inches below the pylorus. Twenty c.c. of practically colorless chyme secured at this point gave a reading, P_H 4.56, in the Hasselbalch cell. On the other hand, McClendon⁷ using modern methods, found the adult human duodenal contents to be slightly alkaline, but he does not give the time of removal in relation to the time of eating. An examination of twenty-three samples of duodenal contents from month-old babies, led him to believe that the infant's duodenum is more acid than the average acidity of their stomachs.

In the jejunum, as in the duodenum, the bulk of experimental evidence seems to point to an acid reaction of its contents. McClendon⁸ passed a Rehfuess tube in a healthy adult a distance of seven feet from the teeth. The average figures for each day showed the contents to become less acid as the tube descended. The greatest acidity was encountered on the first day, P_H 4.7. The nearest approach to alkalinity was recorded on the fifth day, P_H 6.2. Gold electrodes plated bright with iridium were employed in these determinations. During the same year McClendon, Bissell, Lowe, and Meyer⁹ had two other healthy men swallow over seven feet of tubing. The subjects, designated as A and B were fed well balanced diets. Specimens obtained the first day, with both tubes probably in the jejunum, showed a P_H for A, 5.1, for B, 4.5. On the second day A's P_H was 4.9, B's 5.2. During the fourth day several specimens were tested from each of the two tubes. There was a tendency for the material aspirated to become less acid, readings of 6.2 and 6.4 being recorded for B. On the fifth day a specimen from A's tube gave a P_H of 6.5, while B's reading was 5.9. These findings led these observers to express the belief that the alkalinity of intestinal contents increases as one descends from the duodenum toward the ileum. Three years prior to this work Long and Fenger⁶ found the contents to be more alkaline. Four male hospital patients, who were not suffering from any disorder of the alimentary tract, consented to swallow Rehfuess tubes for the collection of intestinal speci-

ment. Subject No. 2 swallowed a tube immediately following a meal consisting of meat, potatoes broiled and milk taken at midnight. At 11 A.M. the tube was some seven or eight centimeters beyond the duodeno-jejunal bend. Collections were then begun and continued with the following results:

P_E	$\frac{4.0}{1.2}$	$\frac{6.2}{2}$	$\frac{6.5}{3}$	$\frac{7.1}{4}$	$\frac{7.1}{5}$
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Subject No. 3 was allowed a diet of meat, potatoes and bread. Upon the day after the tube had been swallowed the tube had descended well beyond the duodenum. A specimen obtained at this time contained much bile and gave a P_E of 5.62. A day later the tube had not moved from its position and there was plenty of bile in the specimen obtained. The P_E of this material was 6.26. Subject No. 4 swallowed a tube and allowed it to drop to the beginning of the jejunum before any collections were made. The first Louis brought up some three hours after finishing breakfast showed the presence of bile. Its P_E was 7.60. The same man, one day later, with the tube still in position and bile present had a P_E of 7.15. These observations appear to place considerable importance upon the presence or absence of bile. Their experience led them to believe that the end of the tube is just below the pylorus; the contents brought up may give a strongly acid reaction whereas, if at a slightly lower level, the pancreatic juice and bile entering at the same point may give a distinctly alkaline reaction because of incomplete mixing with the acid chyme. This of course may be possible but there are conditions which McClendon and his collaborators did not encounter. In the upper small intestine of two two dog, Gratzel and Miller's found no contents more alkaline than P_E 6.01. The average reading for ten dogs on normal diets was P_E 5.90.

On account of the relative inaccessibility with which fistulae are encountered in the lower levels of the small intestine the ileum early became a center for observation. In 1919 Ewald's studied the material which issued from a fistula, probably situated in the lower third of the small intestine. The fluid had a slightly fecal smell. Its reaction was neutral or faintly acid, never alkaline. Two years later MacFayden, Neebha and Schabert obtained material which was continuously voided through a fistula of the lower ileum. This material was also acid in reaction. These workers unfortunately were unable to state their results in terms of P_E . In 1917 McClendon, Shedor and Thomson's saved seven puppies all from the same litter for their study. The dogs were killed on certain arbitrarily selected days and determinations were made with the hydrogen electrode. Their findings were as follows:

AGE DAYS	7	10	11	12	13	14	15
P_E OF ILEUM	5.7	6.75	6.34	6.3	6.1	6.0	6.0

One year later McClendon, Shedor and Karpman made similar observations upon adult dogs fed on a mixed diet of cooked food. The animals were killed at various intervals after feeding. The hydrogen electrode was inserted through punctures in the intestinal wall, and samples of intestinal contents drawn directly into them. Here is a reproduction of their findings:

historic interest. Therefore, only such studies as are pertinent are included, these for the most part having been published in the light of modern advances in the knowledge of hydrogen ions. No attempt has been made to present references in their chronologic order. But so far as possible, some semblance of anatomic order has been observed, beginning with the duodenum.

For many years it was taught that neutralization of the acid chyme took place within a few inches distal to the pylorus, and that the intestinal reaction remained neutral or nearly so thereafter. There is at present, however, considerable experimental evidence to indicate that this is not always the case, that the duodenum may remain acid throughout its entire length. Givazol and Miller⁴ made hydrogen-ion determinations upon the duodena of thirty-two dogs. These animals were fed on a variety of diets. The nearest approach to alkalinity was found in six dogs on a rachitic diet. The average duodenal P_H of these animals was 6.59. The average of ten dogs on a normal diet was P_H 5.91. The determinations were made both electrometrically and colorimetrically. Long and Fenger⁵ studied the reaction of intestinal contents of men. One subject was given an abundant diet of toast, figs, milk, and coffee. The Rehfuß tube was taken soon after breakfast, and allowed to work down through the pylorus. An hour after this the tube was about six inches below the pylorus. Twenty cc of practically colorless chyme secured at this point gave a reading, P_H 4.56, in the Hasselbalch cell. On the other hand, McClendon⁷ using modern methods, found the adult human duodenal contents to be slightly alkaline, but he does not give the time of removal in relation to the time of eating. An examination of twenty-three samples of duodenal contents from month-old babies, led him to believe that the infant's duodenum is more acid than the average acidity of their stomachs.

In the jejunum, as in the duodenum, the bulk of experimental evidence seems to point to an acid reaction of its contents. McClendon⁸ passed a Rehfuß tube in a healthy adult a distance of seven feet from the teeth. The average figures for each day showed the contents to become less acid as the tube descended. The greatest acidity was encountered on the first day, P_H 4.7. The nearest approach to alkalinity was recorded on the fifth day, P_H 6.2. Gold electrodes plated bright with iridium were employed in these determinations. During the same year McClendon, Bissell, Lowe, and Meyer⁹ had two other healthy men swallow over seven feet of tubing. The subjects, designated as A and B were fed well balanced diets. Specimens obtained the first day, with both tubes probably in the jejunum, showed a P_H for A, 5.1, for B, 4.5. On the second day A's P_H was 4.9, B's 5.2. During the fourth day several specimens were tested from each of the two tubes. There was a tendency for the material aspirated to become less acid, readings of 6.2 and 6.4 being recorded for B. On the fifth day a specimen from A's tube gave a P_H of 6.5, while B's reading was 5.9. These findings led these observers to express the belief that the alkalinity of intestinal contents increases as one descends from the duodenum toward the ileum. Three years prior to this work Long and Fenger⁵ found the contents to be more alkaline. Four male hospital patients, who were not suffering from any disorder of the alimentary tract, consented to swallow Rehfuß tubes for the collection of intestinal spec-

The literature agrees in a general way that the intestinal contents are acid in the duodenum, and become progressively less acid on descending the tract. However, there has been little attempt to study the hydrogen ion concentration under controlled conditions. Differences in the reports are, therefore difficult to evaluate. This is particularly true of the work which has been done to show the effect of different foods upon the reaction, then again, the literature published before it was possible to determine the hydrogen ion concentration is of little value for accurate work. It, therefore seemed necessary to determine the hydrogen ion concentration under known conditions, before it was possible to study the various elements that might affect it. Since we started this work, Grayzel and Miller published the results of an almost similar study, but as we were well along on our problem it seemed worth while to complete it.

EXPERIMENTAL WORK

Adult male dogs, with one exception were selected as the animals of choice for the present work. Dogs were chosen, as their alimentary tract seems to simulate the human as closely as that of any animal, as well as for the ease with which these animals can be induced to take different kinds of foods. It was thought wise to use males to eliminate the possibility of pregnancy or estrus complicating the tests. Adult dogs were used because they are more stable than puppies, and it was thought they might be more apt to eat the various diets. The animals were healthy, and weighed between sixteen and twenty three kilograms.

Two animals were fed on a mixed diet of cooked horse meat and white bread. The meat contained only a relatively small proportion of fat. Each dog was given approximately 350 gm of meat and 100 gm of bread daily. Water was allowed ad lib. This constitutes what will hereafter be termed the normal diet. Dog 1 had been on the above regime for several months. Dog 2 was fed the normal diet for two weeks. In addition the tract of Dog 3 a female, was studied and included, as it was essentially normal, although having had both kidneys treated with x rays some time before. The kidneys were delivered through a flank incision, and the surrounding tissues well screened with lead plates so that the rest of the body was entirely shielded. This animal received one half to one pint of milk daily with the normal diet. She had received this diet for a period of several months. Dogs 4 and 5 had been given approximately 350 gm each of cooked horse meat daily for fifteen days. This, with water, constituted their entire diet, and will be referred to as the protein diet. Dogs 6 and 7 were given approximately 200 gm each of white bread, daily, for two weeks. Bread and water was all they received during this period, and constitutes the carbohydrate diet. Dogs 8 and 9 were put upon a diet of fat containing 200 gm of lard. They were also given 100 gm of bread to prevent acidosis. On the morning of the twelfth day of this régime animal 8 refused to take his food and was killed that afternoon. Dog 9, on the other hand took his daily ration readily, and with apparently much relish. He was killed after having remained on the diet for fourteen days. Except for the bread diet the food

was obviously not limited to one food product. But this never occurs in the diets of humans. A marked preponderance of one type of foodstuff, therefore, satisfied the conditions which were to be investigated.

All except Dogs 1 and 3 were killed approximately twenty-four hours after a feeding. Other workers on this subject apparently have killed their animals or obtained their specimens within six hours postebum, and for the most part, material has been obtained within one to four hours. McClen-don and Sharp²¹ in 1919, showed that most foods have an acid reaction, and in general they become slightly more acid on boiling. They found that crushed rabbit muscle gave a P_H of 6.0. The present work was undertaken in an attempt to determine if a permanent change could be produced in the reaction of the intestinal contents by the feeding of protein, fat, or carbohydrate. In order to minimize the effect of the food itself, and at the same time observe a more permanent state of the intestinal contents, it was decided to kill the animals on the day after a feeding, and this was done except for the female (Dog 3) which was killed approximately four hours P. C. Dog 1 was also killed about four hours after a feeding.

The dogs were killed practically instantaneously by intravenous injections of hydrocyanic acid or ether. Dogs 1, 2, and 7 were killed by injecting approximately 15 c.c. of 2 per cent hydrocyanic acid into a hind leg vein. The others were given approximately 20 c.c. of ether in a hind leg vein. Cessation of respiration occurred almost instantly when the full 20 c.c. of ether were injected.

In most instances, the animal's heart was still beating when the abdomen was opened. Various intestinal levels were selected, and immediately tied off between double ligatures to prevent the movement of contents from one portion of the gut to another. Then, the entire intestinal tract, from pylorus to lower rectum, was removed in one piece and washed with tap water. The location of the ileocecal valve was estimated, and a point on the external intestinal wall directly over it chosen as a center from which to measure the distances to the variously selected segments. These measurements were recorded as so many centimeters oral or aboral to the valve. Punctures were then made into the intestinal lumen of each previously selected segment, and the intestinal contents expressed into test tubes containing water of known P_H . It was found impractical to obtain the material under oil. Whenever possible, two specimens were obtained from the same segment, for duplicate determinations. Within a period varying between ten minutes and one and one-half hours, the material was transferred from the test tubes to centrifuge tubes, centrifuged, and the comparatively clear top fluid decanted into the reading tubes and tested colorimetrically.

The hydrogen-ion concentration was determined colorimetrically, as this method gives sufficiently accurate readings for the work in hand. (See Grayzel and Miller⁵)

DISCUSSION OF RESULTS

Table I shows quite clearly that no fundamental changes in reaction occurred with a protein, fat, or carbohydrate diet. This can probably be more readily seen in Chart I. These curves portray graphically the result ob-

tained when the average readings for dogs on the same diet are plotted, using P_H as ordinates and per cent distance from pylorus to ileocecal valve as abscissae. The numerals 1, 2, 3, etc. represent the dog number, e.g., 4 and 5 were both on a protein diet, while 8 and 9 received one consisting chiefly of fat. With the exception of 2 (dog on normal diet killed twenty

TABLE I

DOG NO	DIET	KILLED	DUODENUM	UPPER SMALL INTES TINE	MIDDLE SMALL INTES TINE	LOWER SMALL INTES TINE	CECUM	COLON
1	Normal	4 hr P C	53 P	60 P	66 P	70 P	No spec	61
2	"	24 " "	66 M	67 S	67 S	72 S	68	62
3	"	4 " "	60 P	60 P	61 P	69 P	58	No spec
4	Protein	24 "	59 P	61 P	65 P	65 P	60	66
5	"	24 "	65 M	63 M	66 S	65 S	66	66
6	Carbohydrate	24 " "	63 M	66 M	64 M	72 S	64	68
7	"	24 "	63 M	65 M	67 S	71 S	68	No spec
8	Fat	24 "	64 M	64 P	66 P	67 P	64	64
9	"	24 "	63 M	63 P	65 P	60 P	62	63

P C—Postcibum

P—Plenty of contents

M—Moderate amount of contents

S—Small amount of contents

four hours postcibum) the curves are very well bunched no great fluctuations occurring. It will be noticed that at 10 per cent of the distance from the pylorus to the ileocecal valve there is a difference of less than 0.2 between the highest and lowest P_H values of all curves except 2. At 50 per cent of the distance the maximum difference of any two readings is less than 0.1. From this point down the tract there is a gradual decrease in acidity, the highest point being reached between 80 and 95 per cent of the distance from pylorus to cecum. Curves 4, 5 (protein diet) and 8, 9 (fat diet) reached their height at 80 while 1, 2 (normal diet) and 6, 7 (carbohydrate diet) attain their maximum at 95 per cent. It is our feeling that no particular significance should be attached to the more alkaline readings in the lower ileum for carbohydrate and normal diets as compared with the readings on the other diets. This is thought to be due to minor variations that will be discussed later, and that it is only the result of chance that it occurred this way. Only curves 6, 7 and 2 really extend beyond neutrality. We cannot explain the greater alkalinity found in curve 2 over that of 1, 3 which represents a normal diet. At first thought it might be due to the fact that 2 was killed twenty-four hours after feeding whereas 1 and 3 were killed within six hours after feeding. But on the basis of this explanation one would expect that 2 would more closely approach the other animals killed after a day's fast and curves 1 and 3 would be isolated, which is not the case. In all instances both cecum and colon were more acid than the ileum. There is a general tendency for the cecal content to be more acid than that found farther along the colon.

The outstanding feature of Chart I is the general type of curve, being acid in the duodenum, becoming gradually less acid on descending the tract,

reaching neutrality or even alkalinity in certain instances, at the end of the ileum. The curve then takes a sudden drop to acidity in the cecum. Although the curves are not smooth it is felt that the notchings would disappear if more animals were studied and averaged. Compare Charts I and II for this purpose. There is no evidence from this work, therefore, to suggest that different foodstuffs will permanently change the hydrogen-ion concentration in the tract. It suggests that if feeding certain food substances tends to change the flora, and a change in flora influences the response of the tract, that it does not do so through influencing the P_H of the small intestine.

It seems that the reaction of the intestinal contents should depend on the relative proportions of its ingredients: foods, saliva, gastric juice, pancreatic juice, bile, and succus entericus. As already stated, McClendon and Sharp²¹

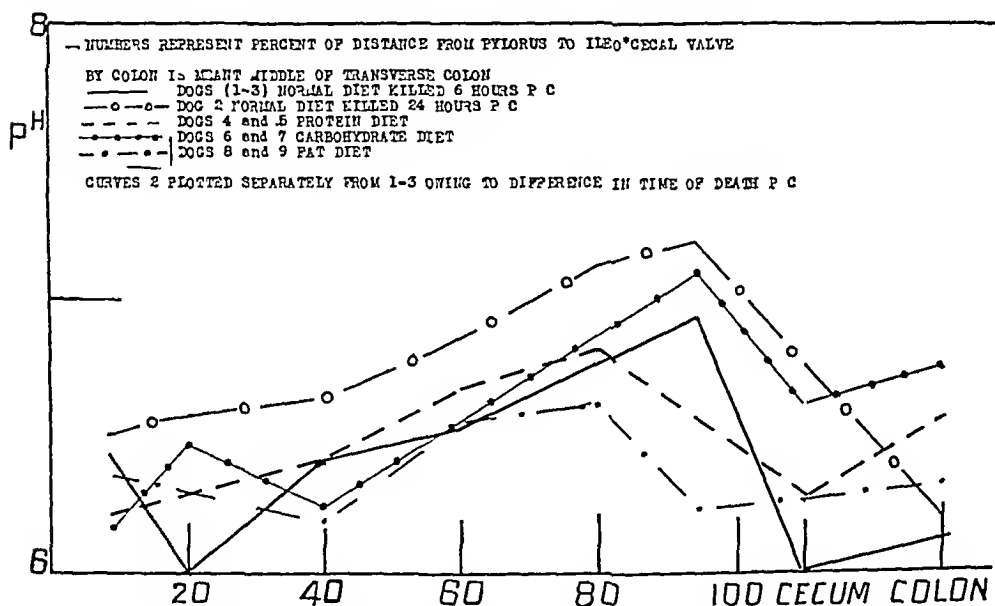


Chart I—The average P_H of intestinal contents of dogs at different levels. Note: Abscissa represents per cent of distance from pylorus to cecum.

showed that most foods have an acid reaction. Saliva is usually slightly alkaline, but gives such a faint reaction in either direction that it may be neglected. Gastric juice is, of course, normally quite acid, being equivalent to 0.46 to 0.58 per cent hydrochloric acid. Starling,²² in his *Principles of Human Physiology*, states that pancreatic juice is markedly alkaline in reaction, varying between N/10 and N/7 sodium carbonate. He further states: "It is therefore about as alkaline as gastric juice is acid, and it will be found that equal quantities of gastric juice and pancreatic juice when added together practically neutralize one another." During 1914, Drury, McMaster and Rous²³ while studying the relation of the reaction of the bile to experimental cholelithiasis found the normal liver bile to be alkaline in reaction, with an average P_H of 8.20. But in the gall bladder, the bile becomes on long sojourn there strongly acid, its P_H ranging between 5.18 and 6.00. Our own

determinations on specimens from the gall bladders of five dogs were somewhat higher than those given above, showing an average of P_H 6.56. Both Howell⁴ and Starling² agree that the succus entericus is distinctly alkaline in reaction. It is therefore not surprising to find that the upper intestinal levels are most acid, becoming gradually less and less, as absorption takes place, with a maximum of alkalinity in the terminal ileum.

Let us consider in more detail the mechanism involved. Food mixed with acid gastric juice enters the duodenum. Here it comes in contact with at least slightly acid bile and pancreatic juice which is not quite so alkaline as gastric juice is acid. Therefore, due to the excess of acidity of bile and gastric juice over the alkalinity of the pancreatic secretion, one should expect to find the upper intestinal tract definitely acid in reaction. But as the intestinal contents move down the tract, undergoing digestion and absorption and

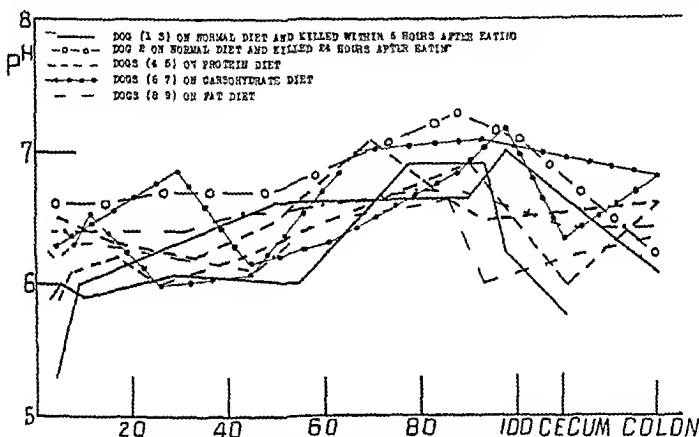


Chart II—Curves to show fluctuations in the P_H of the intestinal contents at different levels. Note: Abscissa represents per cent of distance from pylorus to cecum.

mixing with the distinctly alkaline succus entericus, it is only reasonable to expect the content to become less and less acid. On this hypothesis, material which reaches the terminal ileum should contain relatively less of the acid elements and more of the alkaline ones than at any higher level. By this sort of mechanism we are able to account for the alkaline content observed in the last 15 to 20 per cent of the distance from pylorus to cecum.

Chart II shows quite strikingly the fluctuations which may occur in consecutive intestinal segments. Here again, the numerals, 1, 2, 3, etc., refer to the animal number. Probably the most prominent feature here is the lack of smoothness of the curves. At the time of studying the intestine it was no need that segments would vary a good bit in the amount of material they contained. In most instances the contents would be more alkaline from comparatively empty segments than from full ones. This was really more striking

ing than Chart II would indicate and was noticeable enough for one of us to try and predict whether the readings would be higher or lower than those for the contents of the surrounding segments

As regards the terminal ileum one expects high readings from the comparatively excessive amount of succus entericus, irrespective of the quantity of the material obtained

One other point may be of passing interest For the most part the P_H of the upper tract approaches fairly closely to the P_H of bile Starling's²² observation that the concentration of the pancreatic and gastric secretions are such as might tend to nullify each other, lends some support to the possibility that the bile may be the dominating factor in the intestinal acidity

Clinically there is reason to feel that foods affect the intestinal tract chemically With the possible exception of the dogs fed with fat, there is no

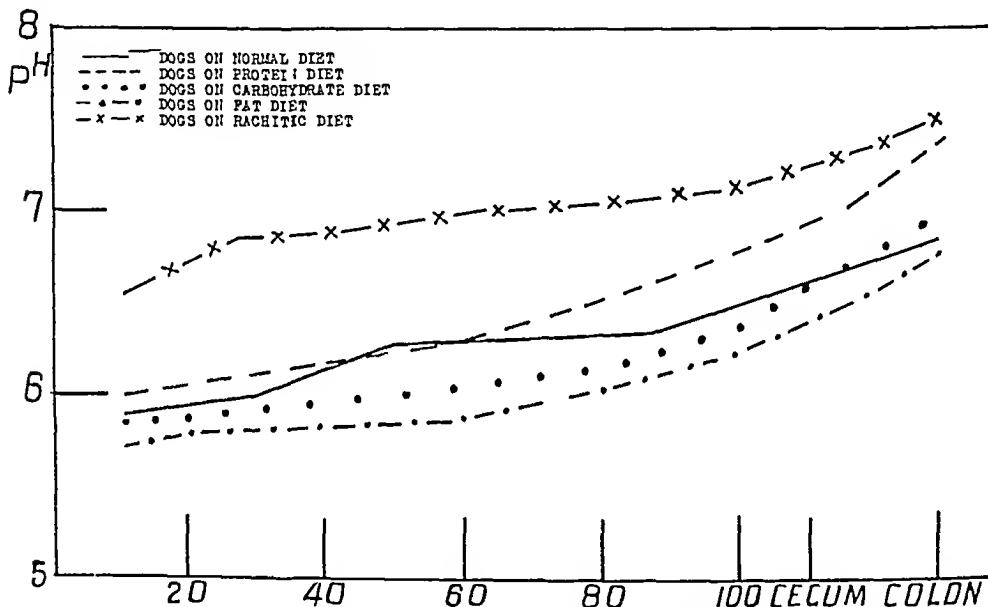


Chart III—Curves plotted from the published table of Grayzel and Miller for comparison with our results. Note: Numbers only approximate. The authors not having given measurements

evidence to feel that the other foods fed affected even temporarily the P_H . It is not likely that the slightly acid reaction which McClendon²¹ has found most foods possess, would have much influence by itself. It is conceivable, however, that foods may influence the P_H by the relative amounts of the different secretions they call forth. Thus fat is known to inhibit hydrochloric acid and stimulate the contraction of the gall bladder. The curves obtained from the dogs fed on fat show a tendency to more acid readings in the ileum than in the case of the other dogs. These dogs were fed excessive quantities of fat, and the intestines were found to contain large amounts of fatty material throughout their entire length. The tendency toward a more acid reaction may, therefore, be due to the fatty acids produced.

The finding of acid contents in the cecum was unexpected. But in every case the cecal contents had a lower P_H than the ileum. It is interesting to

contrast our results from this point of view with those of Gravel and Miller.² They have done essentially the same thing as we did. Having studied the effect of different kinds of food products on normal dogs they determined the effect of feeding a rachitic diet. In dogs on a rachitic diet the P_H was considerably higher than on a normal diet. We have taken the liberty of charting the results from their table in the same general way as our own for the sake of making comparison simpler. Their curves are smoother than ours but agree with ours in the gradual sloping up grade from the acid duodenum to the more alkaline ileum. However our curves show a greater tendency to alkalinity in the ileum than any of theirs except that obtained on a rachitic diet. This might be explained by their having killed the dogs within a few hours postcibum whereas ours for the most part were killed after twenty-four hours. On the other hand the most alkaline reaction in our series occurred in a dog on a normal diet which was killed within six hours. The striking difference between our results are the findings in the large intestine. Their results show a more alkaline colon than ileum whereas the colon of our dogs invariably gave a more acid reaction than in the lower ileum. We are not able to explain these divergent results.

SUMMARY AND CONCLUSIONS

Previous work done on the reaction of the intestinal contents has been unsatisfactory in that much of it was done before accurate determinations of hydrogen ion concentration could be made and because of a lack of controlled conditions.

The present study was undertaken to determine the hydrogen ion concentration of all portions of the tract simultaneously and to determine whether the feeding of a preponderance of one type of food product would change the reaction.

Three dogs were fed diets considered normal for these animals. The others were given a preponderance of protein, fat or carbohydrate for two weeks and then killed instantaneously twenty-four hours after eating and the tract studied immediately.

All showed about the same thing. The P_H ranged from 6.2 to 6.5 in the duodenum. Thence it became gradually more alkaline as it approached the ileocecal valve to become more acid in the cecum. This agrees for the most part with the results obtained by Gravel and Miller except that they found the contents of the cecum to be more alkaline than in the terminal ileum. We have no explanation for these divergent results.

Our results suggest that the reaction of the upper intestine is acid as a result of the hydrochloric acid and bile and become less so as the alkaline succus entericus becomes the more dominant factor. There is no evidence from our results that two weeks' feedings of different food substances influence the P_H of the intestinal tract.

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THE CLINICAL SIGNIFICANCE OF EOSINOPHILIA ON A GENERAL MEDICAL SERVICE*

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REVIEW OF LITERATURE

IT HAS seemed to us that textbooks in their discussions of eosinophilia have been content to list the conditions in which an increase in eosinophiles may occur without giving much idea about their aetnal occurrence. The present survey was undertaken, therefore, to determine if possible, the clinical significance of eosinophilia among the population of a general medical service.

It is proposed first to quickly scan some of the more outstanding features of the eosinophile with such references as may render the original literature easy of access.

In 1864, Wharton Jones¹ first distinguished finely and coarsely granular leucocytes in fresh blood, some of the coarsely granular variety being subsequently shown by Ehrlich² to have specific affinity for eosin, since which time the so called eosinophiles have assumed increasing importance. The subject in general has been quite extensively reviewed by Opie,³ Brown,⁴ Herrick,⁵ Simon,⁶ Schmitz,⁷ Ewing,⁸ Schambert and Strickler,⁹ French,¹⁰ Staubli¹¹ and Zappert.¹²

The eosinophile is common to all mammalian species.³ Most hematologists are agreed as to the origin of eosinophiles from cells of a lymphoid character. The granules of the eosinophiles derived from the bone marrow are of true endogenous origin. They are differentiated gradually from a basophile protoplasm.¹ Numerous investigators have described local heteroplastic development of eosinophiles in various regions in the body.¹³

The average number of eosinophiles in the blood of man is from 2 to 4 per cent, but it has been shown to vary significantly from hour to hour.¹⁴ They are more variable in the blood of children and usually higher in number than in the adult. Six per cent is considered by many a fair average for the normal child.¹⁵ ¹⁶ ¹⁷ Variations in weight of the body seem to cause marked changes in number at least in the guinea pig.³ Fall in weight invariably seems to produce an increase in eosinophiles. There may be cases of so called "constitutional eosinophilia" in which the eosinophiles reach as high a value as 66 per cent in an otherwise apparently healthy man. These cells may also be high in the patient's blood relations.¹⁶ At present we are unable to generalize as to the mechanism of the mobilization of eosinophiles. One of the most useful theories states that anaphylaxis and eosinophilia are usually coincident.³⁴ ³⁵ ³⁶ ⁷⁰ Actually this is often found to be the case.

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In diseases of parasitic origin, especially the animal parasites, eosinophilia is the rule. *Oxyuris*,¹⁷ *Asearis*,^{18, 19} *Taenia saginata*,²⁰ *Echinococcus*,^{21, 22} *Trichina*,^{23, 24} *Uncinaria*,^{25, 26} *Filaria bancrofti*,^{27, -8, 28a} *Bilharzia*,²⁹ *Dibothriocephalus* and *Taenia solium*³⁰ usually show moderate eosinophilias (8 to 20 per cent). Many others show inconstant eosinophilia such as the *Leishmania* and *Trypanosomes*. Amebic dysentery often produces an increase in eosinophiles whereas in the bacillary type they decrease in number.³¹ Many of the protozoa and some of the cestodes do not show an increase in the eosinophile count.

Some skin diseases induce eosinophilia, especially dermatitis herpetiformis, bullous dermatitis, pemphigus, scabies, mycosis fungoides, herpes iris, erythema bullosum, psoriasis and prurigo.^{32, 32} In eczema it is only occasionally found.³

Asthma and hay fever typically bring about a very definite increase in eosinophiles.^{17, 33, 34, 35, 43, 72, 73} Rheumatic fever commonly shows an increase,^{36, 37} migraine at times,³⁸ also Quincke's edema,³⁹ and serum sickness.⁷⁵

It has been repeatedly proved that foreign proteins, such as egg albumin,^{40, 41, 42} horse serum^{40, 43} and tuberculin⁴³ produce eosinophilia.

Chronic bronchitis with emphysema at times may be differentiated from asthma on the basis of the eosinophile count inasmuch as the former seldom induces any marked increase in their number.⁵

Scarlet fever seems to be the only acute exanthem in which a marked eosinophilia is found. Eosinophilia is believed to be a favorable sign, disappearance or a marked fall indicating a serious prognosis.^{44, 45} In diphtheria, eosinophiles are not ordinarily increased unless a foreign protein is introduced, such as antitoxin.

In the case of tuberculosis, especially of the intestinal variety, there may be a marked eosinophilia,⁴⁶ however, in tuberculosis of the lung an absence of eosinophilia is often seen. With suppuration and cachexia they may reappear.⁸ There may be a high eosinophile count in leprosy.^{46, 47} It is claimed that paratyphoid B may be differentiated from typhoid by the presence of eosinophiles in the former disease.⁴⁸ In general, it can be said that following most infections with a return of the leucocyte count to normal, there may be a moderate rise in the eosinophiles—the so called "reactive" eosinophilia. In nearly all forms of acute polynuclear leucocytosis an absence of eosinophiles is found. Scarlet fever, acute rheumatic fever, and malaria at times furnish notable examples.

Malignant tumors rarely produce a high eosinophile count.^{8, 49, 50} Tumors of the colon⁷¹ and cervix⁵¹ may produce both local and general eosinophilia.

The proportions of eosinophiles in leucemia are generally within normal limits, but their total number is greatly increased. Eosinophilic leucemias have been reported showing remarkably high counts.^{52, 53, 54, 55, 56} Splenomegaly seems very usually associated with this type of leucemia. In some cases of Hodgkin's disease, the eosinophiles may reach a high level.^{57, 58, 59}

Local eosinophilias have been quite extensively reported, especially pleural,^{60, 61, 62} intestinal,^{63, 64, 65} and skin exudates.⁶⁶ Rickets, osteomyelitis, osteo-

malacia, malaria, Addison's disease, gonorrhea, varicella chlorosis, echinococcus disease, Hodgkin's disease and uremia occasionally create an eosinophilia

It is interesting to note that under the liver treatment for pernicious anemia there is a progressive rise in the eosinophile count²⁴

CLINICAL DATA

The unit histories of 13,340 patients who entered the Presbyterian Hospital between March 30, 1923, and October 12, 1927, were examined. Of these, approximately 40 per cent, or about 5,500 patients, were medical cases and these form the basis of the present report. All these cases had at least one complete blood count including a differential leucocyte count from a stained smear. Furthermore, on admission, stool examination including a search for ova and parasites was done in practically all cases routinely. In case an eosinophilia was discovered in the blood, several careful searches for intestinal parasites or their eggs were usually made.

Two factors prevent this survey from being entirely representative, no cases of the acute exanthemata are admitted to the hospital, and few dermatologic cases are seen. Moreover, it is entirely possible that occasional increases in the eosinophiles that are more or less transitory may have been missed by too infrequent counts, especially the so called "reactive" eosinophilias occurring in postfebrile states after the return of the total leucocyte count to normal.

By an eosinophilia we mean a condition in which there is 5 per cent or more of eosinophiles in the blood thereby accepting 4 per cent as the upper limit of normal. The hopelessness of such an arbitrary division between the significant and insignificant is obvious. However, in the facts presented below, we shall endeavor to point out those counts that might be misleading, i.e., those in which the eosinophiles are only 5 per cent, but which are included in our list as eosinophilias.

Diseases of the blood, such as anemias, leucemias, purpura, and hemophilia were excluded from this summary.

PARASITIC INFECTION

There were 31 cases due to infection with parasites. Of these, 15 were cases of trichiniasis. In this condition as is well known eosinophilia occurs with great constancy. Eosinophiles may form the majority of the circulating leucocytes reaching as high as 81 per cent in one of our cases, while in only one case was the count constantly below 15 per cent. It is interesting to note that the total white count is not necessarily high, for in 6 of this series, the leucocytes were less than 10,000 per cmm. In general the total count averaged 12,000 to 15,000 with 20, 30, or 40 per cent eosinophiles. Occasional counts were recorded as high as 28,000 and 32,000.

A diagnosis of amebic dysentery was made in 5 cases. In these, the eosinophiles were low (5, 5, 6, and 7 per cent), with the exception of one case in which the eosinophiles ranged from 14 to 62 per cent.

A miscellaneous group of 11 cases included ascaris, two (11 per cent, 19

to 22 per cent eosinophiles), *dibothriocephalus latus*, three (5 per cent, 6 per cent, and 4 to 17 per cent), *uncinariasis*, usually with *trichuris trichura*, three (10 per cent, 13 to 24 per cent, 10 to 33 per cent), *taenia saginata*, one (8 per cent), *oxyuris vermicularis*, one (7 per cent), *filariasis*, two (9 per cent, 10 to 12 per cent)

DISEASES OF LUNGS AND UPPER RESPIRATORY TRACT

Sinusitis—Three cases diagnosed as sinusitis showed eosinophilias of 5 per cent, 8 per cent, and 8 per cent respectively. The total leucocytes were normal in all cases. Four additional cases of sinusitis, three in connection with asthma and one with a generalized acute upper respiratory infection had an increase in eosinophiles.

Acute Pharyngitis, Tonsillitis, Bronchitis, etc—There were 13 cases. Eosinophilia was rarely striking (5 per cent—4 cases, 6 per cent—4 cases, 7 per cent—2 cases, 9 per cent—2 cases). In one case of acute follicular tonsillitis, the eosinophiles were on one occasion 15 per cent and on another 18 per cent.

Lobar Pneumonia—Seven cases. Eosinophiles ranged from 5 per cent to 7 per cent. The eosinophilia occurred in every case as a "reactive" phenomenon, i.e., with a return of the leucocytes to lower or normal levels.

Bronchopneumonia—Six cases. Range 5 per cent to 8 per cent. In this condition, eosinophilia was less often a "reactive" phenomenon and in the majority of cases occurred at the height of the disease.

Asthma, Chronic Bronchitis, Emphysema and Bronchiectasis—Thirty-eight cases. The diagnosis of asthma alone was made in 17 cases. In these cases the eosinophiles ranged from 5 per cent to 18 per cent, averaging 10 per cent. One interesting case was that of an asthmatic with 6 per cent eosinophiles who had a bout of acute rheumatic fever. With the accompanying leucocytosis the eosinophiles dropped to 1 per cent and later rose again with the return to normal leucocyte count. The diagnosis of asthma occurred in combination with others of the group in 11 cases.

The diagnosis of chronic bronchitis alone was made in two cases (5 per cent, 6 per cent) and with others in the group in 10 cases (range 5 to 13 per cent, average, 8 per cent). In one case chronic bronchitis and syphilis were associated.

Emphysema was diagnosed alone in two cases (5 per cent and 10 per cent) and in combination in 7 cases (6 per cent to 9 per cent).

Bronchiectasis occurred in 5 cases: once alone (5 per cent), twice with others of this group (5 per cent and 7 per cent) and twice in luetics (5 per cent and 6 to 11 per cent).

Chronic Tonsillitis—In 3 cases so diagnosed the eosinophiles were 5 per cent, 5 per cent, and 9 per cent.

Serofibrinous Pleurisy—Five cases (5, 6, 8, 8, and 13 per cent). In one case the patient was luetic.

Pulmonary Tuberculosis—Five cases occurred with eosinophiles of 5 or 6 per cent. In two additional cases (6 per cent and 8 per cent) tuberculosis and syphilis were associated.

RHEUMATIC FEVER GROUP

In this group are included cases of acute and chronic rheumatic fever, chorea and rheumatic heart disease. There were 40 cases. Two of these patients had erythema multiforme and one case was luetic. Eosinophilia was inconstant even in the same patient and did not seem to have a definite relation to the total leucocyte count although in some cases it was definitely of the "reactive" type. The usual degree of eosinophilia was from 5 to 9 per cent. Six cases were 10 per cent or above the highest being 19 per cent.

In one remarkable case there were no eosinophiles until the development of a skin lesion suggesting pellagra, whereupon the eosinophiles rose rapidly to 12 per cent to 24 per cent and to 40 per cent associated with a rise in the total leucocyte count to 19,000. Six weeks later the leucocytes were 11,000 and the eosinophiles 3 per cent and sometime later 19,000 and 12 per cent.

"DEGENERATIVE" DISEASES

In this group are included chronic multiple arthritis, general arteriosclerosis, chronic nephritis, and hypertension. No attempt has been made to further subdivide this group. These were 28 cases. In this group are included 3 cases who had also chronic cholecystitis, one with syphilis, one with hay fever, and three with diabetes. As a rule the eosinophilia was not high—about 5 to 8 per cent.

HYPERTHYROIDISM

There were eight cases with a range of from 5 per cent to 9 per cent.

DERMATOLOGIC CASES

This is in no wise a representative group as patients with skin lesions are treated only as outpatients with few exceptions. There were 15 cases in this group. Of these, eight were miscellaneous conditions with eosinophile counts of from 5 to 19 per cent. An interesting case was one of leucoderma, urticaria, chronic bronchitis, and asthma. The patient repeatedly had an eosinophilia of 33 to 36 per cent.

Six cases of arsenic poisoning during antiluetic therapy formed a conspicuous group. The eosinophilia in these cases was 6 per cent, 7 to 9 per cent, 13 per cent, 18 per cent, in one case 24 to 28 per cent, and in the sixth case the eosinophiles reached 65 per cent of the total leucocytes, which numbered 12,000 per c mm.

SYPHILIS

An eosinophilia of from 5 to 17 per cent was present in ten cases in which the primary diagnosis was syphilis. In addition, syphilis was present in 12 of the above mentioned cases and may have been an important factor.

MISCELLANEOUS

There were 84 cases in this group. In a great variety of conditions represented by only one case, an eosinophilia of 5 per cent or even of 6 per cent occurred, often transiently. We believe that this is casual and of no signifi-

cance and these cases are accordingly not treated in detail here There were 42 such cases The remaining 42 may be listed as follows

TABLE I

DIAGNOSIS	NO CASES	% EOSIN	DIAGNOSIS	NO CASES	% EOSIN
Chronic Colitis	6	6-11	Carcinoma, liver	2	6-11
Acute Colitis	1	10	Lymphosarcoma	1	7-10
Periarthritis nodosa	2	8-17	Pylitis	4	5-15
T B of male genitals	1	7	Nephrolithiasis	1	6-12
Gastroparesis	1	7	Hem Staph abscesses	1	7
Undiagnosed condition of abdomen	1	10	Chronic osteomyelitis	1	9
Ulcer of duodenum	6	5-8	Scarlet fever	1	7
Constipation	1	10	Sprue	1	8-27
Anxiety neurosis	1	8	Lead poisoning	1	5-8
Angioneurotic edema	1	14	"Secondary anemia"	1	8
Idiopathic epilepsy	1	7	Thromboangitis oblit	1	10
Paralysis agitans	1	7	Dysmenorrhea	1	
Carcinoma, rectum	1	12	Drug unknown	1	14-20
			Drug unknown	1	73

COMMENT

Parasitic infestation accounted for 10 per cent of our cases of eosinophilia and of these half were trichinosis This group included some of the highest eosinophilic counts, although in amebic dysentery the eosinophilia is uniformly slight The highest count in our series was in a case of trichinosis, 81 per cent

In infections of the respiratory tract occasional cases of uncomplicated sinusitis exhibited persistent eosinophilia An occasional case of lobar pneumonia showed a "reactive" eosinophilia Probably a great many such reactions were missed because of their transitory character In contrast to lobar pneumonia, bronchopneumonia may show an eosinophilia at the height of the disease with a considerable leucocytosis The chronic pulmonary affections (chronic bronchitis, emphysema and asthma) were well represented in this series accounting for about 13 per cent of the total

The rather high incidence of eosinophilia in the rheumatic fever group was a surprise to us There were 40 cases (13 per cent) in this series The eosinophilia was often very transient in the individual patient and while often of the "reactive" type was by no means constantly so Further work is in progress by one of us to determine what relation the appearance of an eosinophilia has to the various phases of the disease

Nearly 10 per cent of the total number were cases of hypertension, chronic nephritis syndrome The eosinophiles while not striking (usually 5 to 8 per cent) were generally persistent

Not infrequently an eosinophilia is found in cases of hyperthyroidism, syphilis, chronic colitis, duodenal ulcer, and pyelitis Eosinophilia is common in arsenic poisoning with skin manifestations

In fully 40 per cent of the cases in the present series the eosinophilia occurred in such a small percentage of the cases of a given group or came so narrowly within our arbitrary definition of eosinophilia that we deem it of no diagnostic importance

It will be seen in the above that eosinophilia occurred in the majority of cases in our series in conditions that theoretically, at least, may be related to allergy. The relation of parasite to host, for example is little understood and may be allergic. In pneumonia a definite antigen antibody mechanism is at work. Conditions of chronic infection may belong to this group. The recent work of Swift and others tends to place rheumatic fever upon an allergic basis. It is in these conditions that eosinophilia is likely to occur. Its exact relation to allergy is not known and much interesting work remains to be done in this field.

SUMMARY

1. An eosinophilia of 5 per cent or more occurred in 300 patients among 5500 general medical cases, all of whom had complete blood counts and stool examinations. The small number of dermatologic cases seen and the entire absence of scarlet fever patients prevent this series from being wholly representative. Cases of obvious blood diseases were excluded.

2. Of the 300 cases of eosinophilia in this series, 10 per cent occurred in parasitic infestation, 13 per cent in rheumatic fever, 13 per cent in chronic pulmonary disease (chronic bronchitis, emphysema, asthma), 10 per cent in the chronic nephritis general arteriosclerotic group. In fully 40 per cent the eosinophilia occurred in isolated cases of various conditions and had no diagnostic significance.

It is suggested from this series that eosinophilia may be a part of the phenomenon of allergy.

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ON THE PHYSIOLOGIC ACTION OF PRESSOR X (COLLIP)*

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PROFESSOR J B COLLIP¹ (1928) has recently prepared extracts containing a pressor substance, from various animal tissues and organs. At his request, we have investigated the physiologic action of several of these ex-

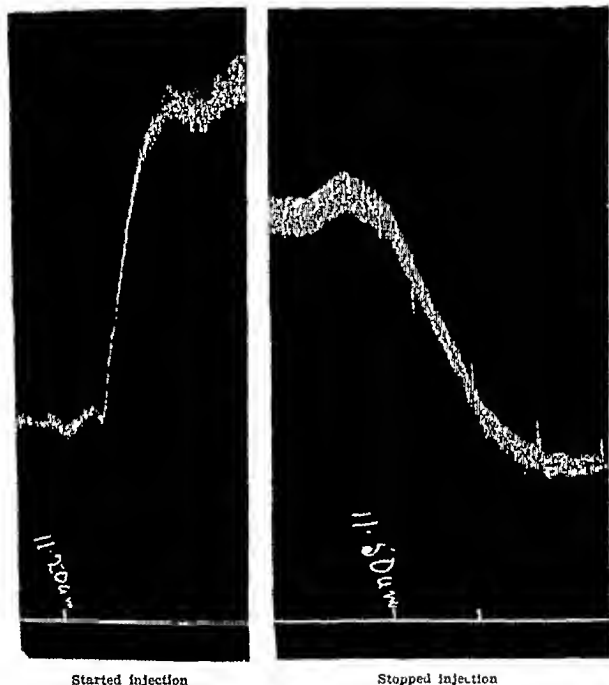


Fig 1—Dog 3 Blood pressure Three c.c pressor X testes (1) in 150 c.c saline intravenously

tracts, and it seems advisable, for the guidance of others, to place on record a brief statement of the chief results. Like epinephrin, this substance, which one may call pressor X, causes a marked rise in blood pressure accompanied

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¹Collip J B Am Jour Physiol 1928 lxxxv 390

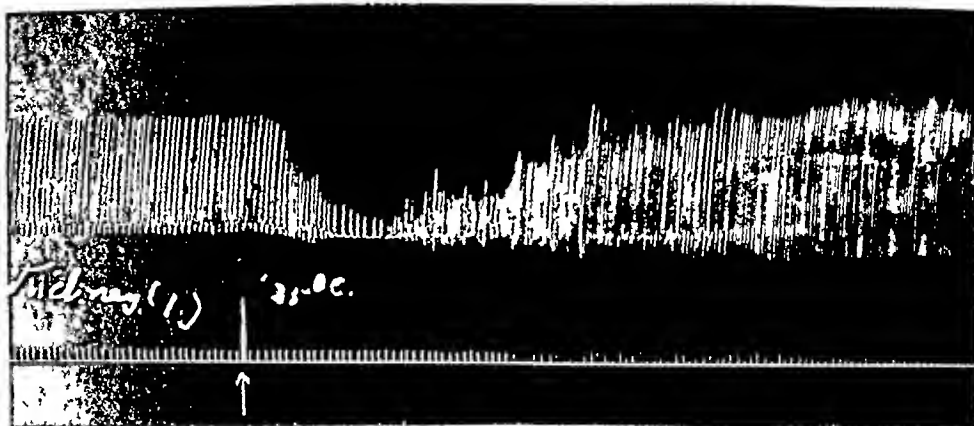


Fig 2 —Perfused rabbit heart Pressor X (kidney) added to perfusion fluid

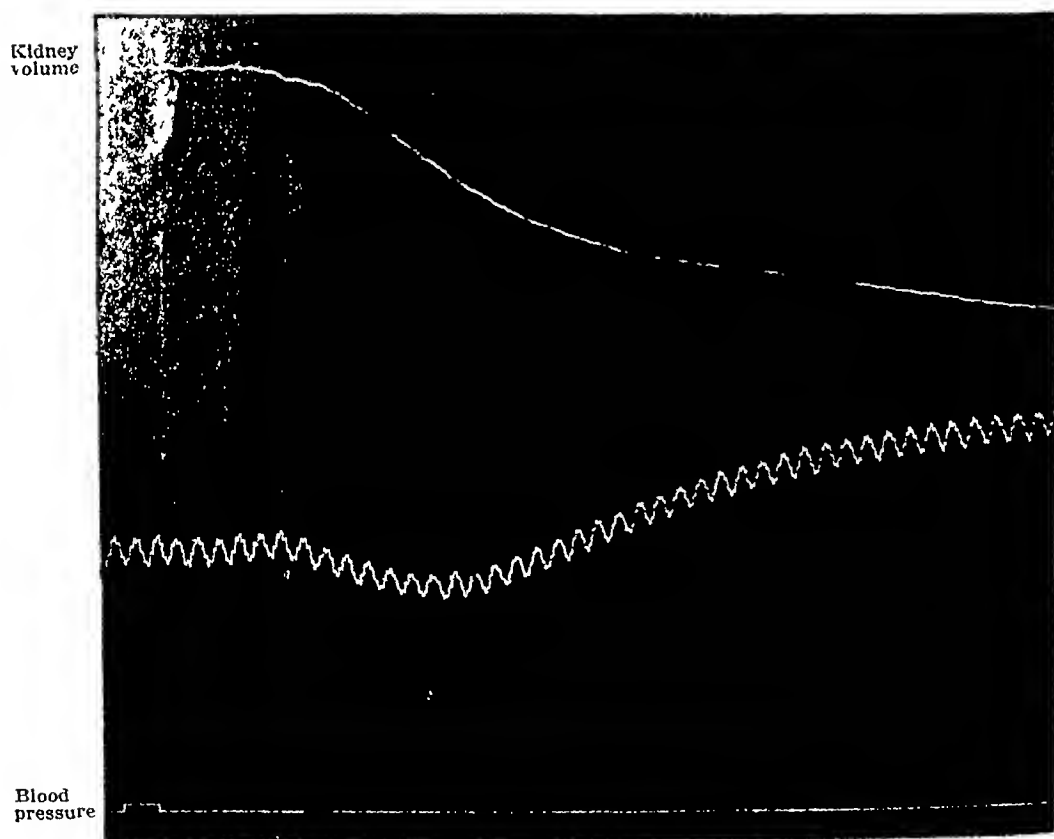


Fig 3 —Dog 2 Pressor X intestine

by an acceleration of the heart rate and a diminution in kidney volume, but its action on the intestine, uterus, and the blood sugar is quite different
 Injected continuously into the veins of dogs anesthetized with ether or

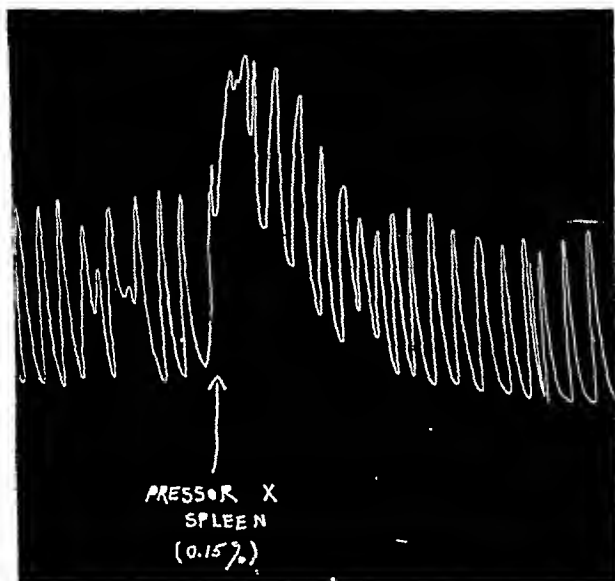


Fig 4 —Rabbit intestine.

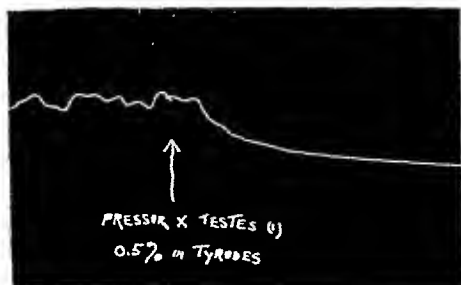


Fig 6 —Virgin guinea pig uterus

amytal, the substance caused a marked rise in blood pressure, which fell to the initial level or below it, immediately the injection was discontinued (Fig 1) The rise in blood pressure was usually accompanied, in the intact animal, by

a slight acceleration of the heart. This acceleration was also observed in the isolated perfused mammalian (rabbit) heart, this being preceded by a slowing and lessening of the beats (Fig 2). With the increased blood pressure there occurred a fall in kidney volume, as shown in Fig 3. This tracing also shows the initial fall, preceding the rise in blood pressure, due to the presence of a depressor substance (probably histamine). It should be pointed out that this was observed only in extracts of intestine, but not in the other extracts. Intravenous injection of pressor X into decapitate cats produced pressor

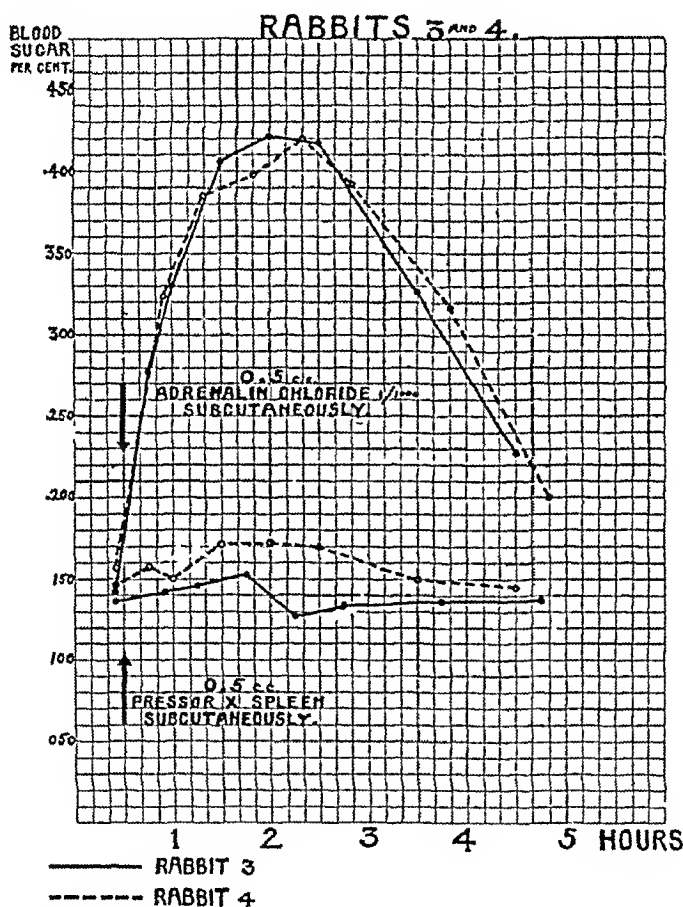


Fig 6—This shows the striking difference between pressor X and epinephrin as regards their effect on the blood sugar. The pressor extract used in this experiment had been previously shown to exert about the same influence on the blood pressure as a 1/1000 solution of adrenalin chloride (Parke Davis).

effects quite similar to those obtained in intact animals. In decapitate eviscerated cats and in an eviscerated dog, it produced a definite rise in blood pressure, though not so marked as in the intact animal, indicating, therefore, a constriction of the blood vessels of the muscles and other extraabdominal tissues.

When added to the nutrient fluid, pressor X caused only a slight and transient increase in the tonus of the isolated rabbit intestine (Fig 4). It did not

TABLE I

RABBIT	EXTRACT	ROUTE	NORMAL BLOOD SUGAR PER CENT	HIGHEST BLOOD SUGAR PER CENT WITHIN 4 HOURS
1	Intestine	Subcutaneous	0.181	0.210
2	"	"	0.153	0.197
3	Spleen	"	0.138	0.153
4	"	"	0.147	0.173
5	Testes	Intravenous	0.182	0.202
6	"	"	0.183	0.177

not revive the contractions inhibited by epinephrin. It inhibited the rhythmic contractions of the similarly observed virgin guinea pig uterus (Fig 5).

Perhaps the most interesting contrast between pressor X and epinephrin is with regard to its effect on the blood sugar level. Compared to the effects

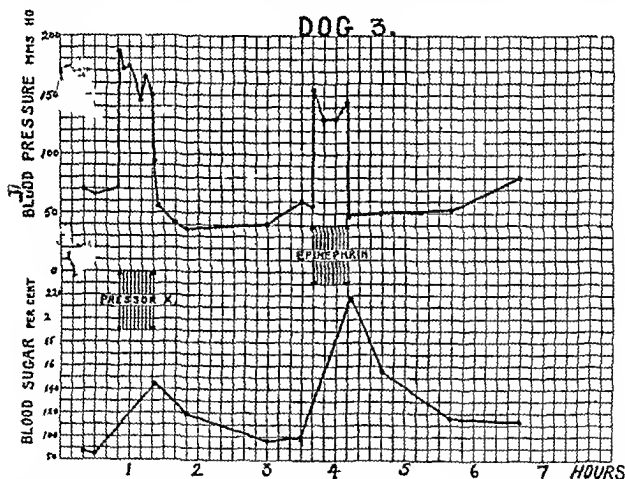


Fig 7—Continuous intravenous injections of 3 cc pressor X tests (1) in 150 c.c. saline and 15 c.c. 1/1000 adrenalin chloride (Parke Davis) in 75 c.c. saline were administered over a period of half an hour as indicated.

of similar doses (as judged by the pressor effect) of 1/1000 adrenalin chloride (Parke Davis), the influence of the pressor extracts on the blood sugar was relatively insignificant. (Fig 6) Injected intravenously into dogs, the pressor substance had a somewhat greater influence on the blood sugar level. This effect, however, was still very much less than that produced by doses of epinephrin causing a rise in blood pressure of the same magnitude (Fig 7).

Since the active principle responsible for this effect has not been isolated, the only method available for the approximate determination of dosage was comparison of the extent of the rise in blood pressure produced by injecting, into animals, comparable quantities of the two substances. Before further

work can be done, it will be necessary to evolve more accurate methods of assay, but since the problem cannot be continued with at the present time, it seemed to us important that the above facts be placed on record

132 NASSAU STREET

THE INFLUENCE OF P_{H} ON THE SELECTIVE BACTERIOSTATIC ACTION OF GENTIAN VIOLET ON MEMBERS OF THE COLON GROUP OF ORGANISMS*

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IN A PREVIOUS paper† on the use of gentian violet in water analysis, we reported a marked difference in the toxicity of gentian violet for certain strains of *B. coli*. Winslow and Doloff published a similar report, 1922, which indicated a somewhat lower toxicity for certain of these strains than we found for our strains. Hall and Ellefson reported the satisfactory use of this dye in much lower dilutions in milk and water examinations. In the previous work for obvious reasons we kept the reaction of the media constant, varied the strength of the dye, and added the dye to the media in which the organisms were incubated. The bacteriostatic action of basic substances, dyes as well as other compounds and some of the factors which influence this action has been reported by Klinger, Davis, Norton and Davis, Churchman, Smith, Kunnwerde and Pratt, Beckwith, 1921, Browning, Gulbransen and Henneway, 1920, and others. The work of Stearns and Stearns, 1924, on the "Chemical Mechanism of Bacterial Behavior with Special Reference to Bacteriostasis and Gram's Reaction" has offered chemical explanation of this phenomena. They presented data to show that the behavior of bacteria toward a dye is largely determined by the nature of the bacterial protein, this being an ampholyte combining with acid dyes on the acid side of their isoelectric point, and with basic dyes on the alkaline side. In our work we found that two closely related gram-negative organisms exhibited a marked difference in their sensitiveness to the bacteriostatic effect of a basic dye. Stearns and Stearns again in 1924 sighted still more data which tend to show that the phenomena of bacteriostasis is a purely chemical one. Besides the chemical composition of the dye they point out the importance of (1) composition of the media, (2) mass effect of bacteria, and (3) reaction of the media. Basic substances in the media would presumably aid the basic dye and increase its bacteriostatic effect and acid substances decrease it.

In our previous work we added the dye directly to the media. We, therefore, decided to set up a series of experiments in which the dye and

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bacteria would be brought together outside of the media in which they were to be grown, and that we would vary the hydrogen ion concentration of the mixtures (dye and bacterial suspension) and the length of time of exposure of the bacteria to the dye. We hoped in this way we might be able to demonstrate the effect, if any, of the P_H on the sensitiveness of these two strains of different groups of *B. coli* to gentian violet and to exclude all factors influencing this difference except the character of the bacterial protein.

EXPERIMENTAL WORK

We experienced some difficulty in selecting a suitable buffer mixture that would permit a homogenous solution of the dye and would not effect the organisms. Clark's (1917) buffer solutions were first tried, but owing to the fact that these salt solutions invariably precipitated much of the dye, and the inhibiting effect of the salts were an unknown quantity, they were abandoned. For similar reasons Sorenson's (1912) buffer solutions were not employed. Witte's peptone was finally decided upon. It was found that a 1 per cent solution of this peptone could be easily adjusted to any desired P_H value by the addition of N/10 HCL or N/10 NaOH. It was also found that the gentian violet appeared to remain in true solution in this buffer mixture at P_H values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0, the values used in this work.

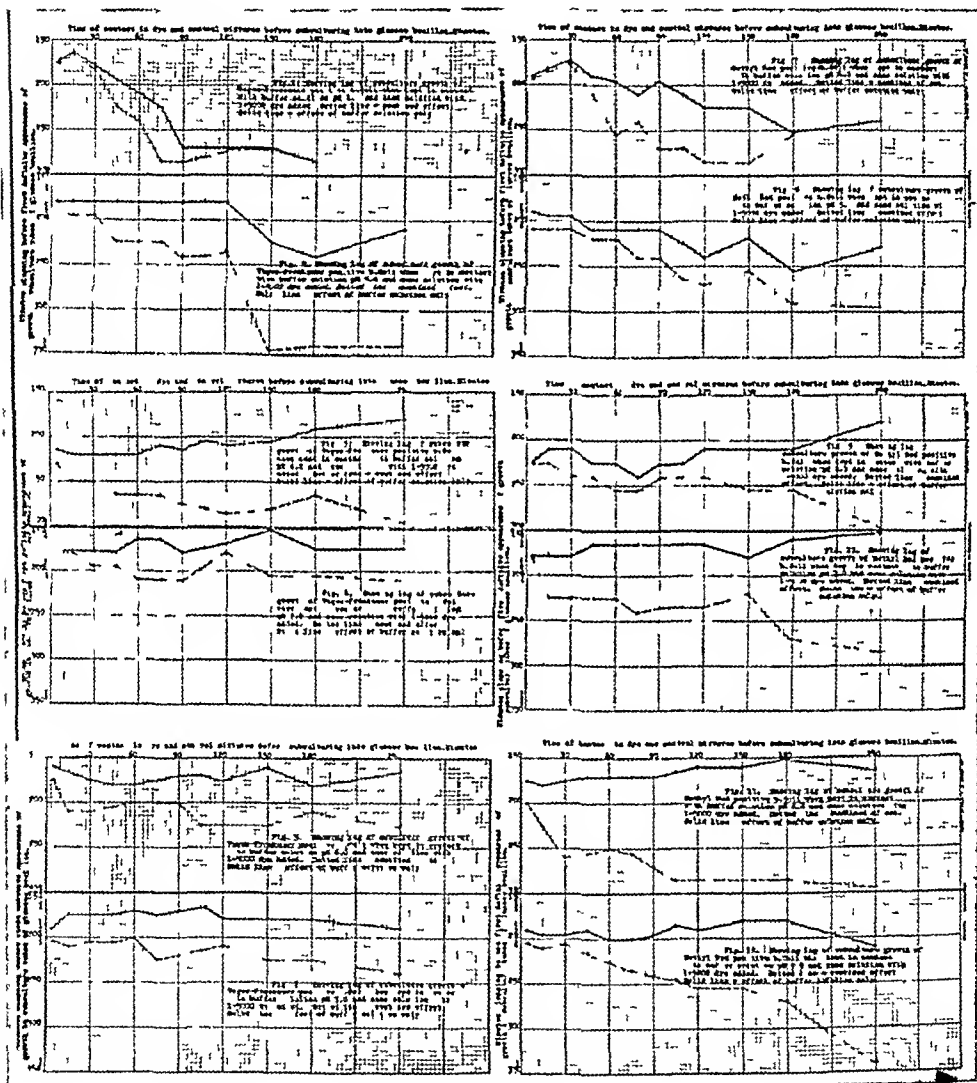
The peptone solution was made by dissolving 10 grams of Witte's peptone in 1000 cc of distilled water. This solution was boiled and filtered. The adjustment to the desired P_H value was made by addition of N/10 HCL or N/10 NaOH and comparison with standard buffer solutions. The indicators used were Clark's series except for the P_H value 4.0 and in this case Methyl orange (La Motte) was substituted for Brom phenol blue. These buffer solutions were adjusted, boiled, filtered and given a final check before use in the experiments.

The dye concentration of 1:5000 (National Aniline Chemical, Chemical Co. "Gentian Violet 6B" lot number 2033) was used throughout these experiments. This strength was used because we desired to get a definite inhibitory action.

The exact procedure for exposing the organisms to the action of the dye buffer mixtures was as follows:

"Methyl Red positive organisms strain I and Voges Proskauer positive organisms strain 18 of our previous work (1926) were the organisms used. The suspensions of organisms were obtained by washing a twenty-four hour growth from an agar slant with 2 cc of sterile salt solution. From this suspension 1 cc was pipetted to 15 cc of the buffer mixture of the desired P_H value. This constituted the standard suspension for the experiment. To 40 cc of the buffer solution of desired P_H value was added 1 cc of 1 per cent aqueous solution of the gentian violet and 10 cc of the standard suspension. This is the dye buffer contact mixture. For the buffer contact mixture without dye hereafter known as 'control' 8 cc of the desired buffer mixture was mixed with 2 cc of above standard suspension. The temperature of the mixture during the time of contact with bacteria was approximately 22° C.

At the end of definite periods of time after the preparation of these contact mixtures, viz, 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, and 240 minutes, subcultures were made into glucose bouillon by withdrawing 0.1 cc from the contact dye mixtures and the controls. These subcultures were incubated at body temperature and observed for the first slight but definite

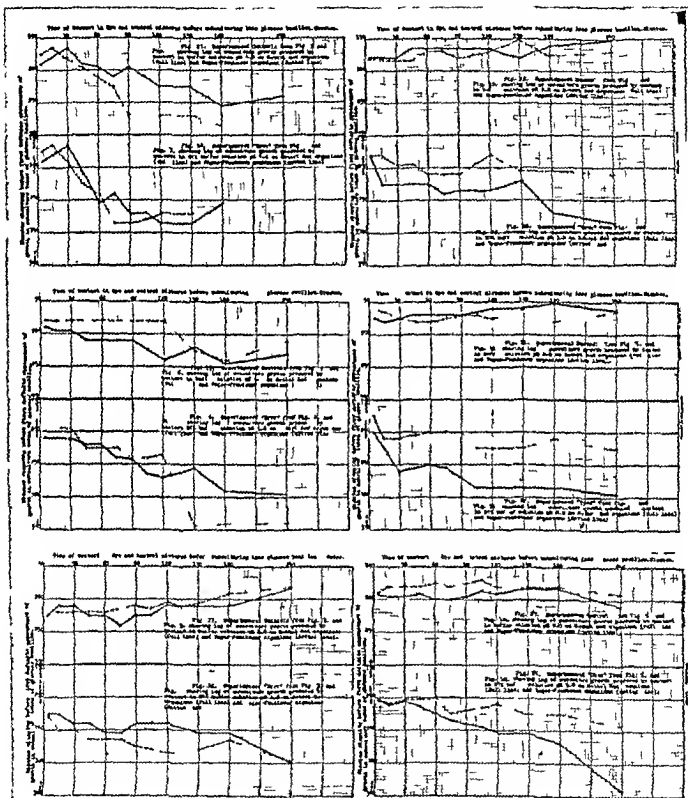


Figs 1-12

appearance of turbidity. This method of showing the lag of subculture growth is accurate to within ten minutes. The results of these experiments are graphically shown in the charts, Figs 1 to 12, inclusive. Comparison of the effect of the dye and control mixtures on the growth of the two types of organisms are shown in Figs 13 to 24, inclusive.

DISCUSSION

Comparison of Figs 1 to 6 show that the greatest effect of the dye is evidenced at P_H 50 and 60 for *Voges Proskauer* organisms while at P_H 40 the dye effect is closely approached by the buffer solution without the dye



Figs 1 to 6

It is seen that at P_H 60 the dye has its greatest effect upon *Voges Proskauer* organisms for at this P_H the buffer solution is shown to have practically no effect

Comparison of Figs 7 to 12, inclusive show that the greatest inhibitive effect of the dye is evidenced at P_H 70 and 80 for the *Methyl Red* organisms. The strongest inhibition is shown in Fig 11 at P_H 80

For both types of organisms it is seen that the buffer showed no effect on subculture growth at P_H values of 6.0, 7.0, 8.0, and 9.0

In Figs 13 to 24, inclusive, is shown the first twelve curves superimposed in their respective order to show the effect of buffer and of dye buffer on the two types of organisms, at each P_H value. Here again it is seen that the effect of the dye on Voges-Proskauer organisms is greatest at P_H 5.0 and 6.0 and greatest on the Methyl Red organisms at P_H 7.0 and 8.0. In the buffer solution alone the organisms behave quite alike except that the aversion of the Methyl Red type to alkali and the Voges-Proskauer type to acid is evidenced to a slight degree.

SUMMARY

Using buffered solutions which at P_H values above 6.0 showed no inhibitive action on the growth of the organisms, it was possible to demonstrate the influence of P_H on the selective bacteriostatic effect of gentian violet on certain strains of *B. coli*. By these experiments we have eliminated the interfering action of the products of bacterial growth in media when the dye is added to the media. It is also apparent that the P_H values to which these organisms were exposed to the dye determined the strength of the inhibitory action of the dye, and that the growth of two members of the colon group of organisms (methyl red positive and Voges-Proskauer positive) are affected greatest by the dye at different degrees of acidity. Following Stearn's explanation of the bacteriostatic action of dyes our results indicate that there is a wide difference in the isoelectric range of the bacterial protein of two closely related strains of gram-negative organisms, and that the gram-negative reaction covers a wide range of P_H values.

These results also indicate that gentian violet has its most selective bacteriostatic effect on members of the colon group when acting in a solution buffered to a P_H of 8.0, and the action is markedly selective in this group between P_H 7.0 and 8.0.

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THE OCCURRENCE OF PARATYPHOID AGGLUTININS IN SERA TESTED FOR TYPHOID AGGLUTINATION*

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PRACTICALLY all serologists are agreed upon the fact that the macroscopic method of conducting the agglutination test for the presence of typhoid agglutinins is the method of choice and, whenever the character of the specimen permits this technic, it is customary to test for the presence of paratyphoid agglutinins as well.

Whenever paratyphoid as well as typhoid agglutination occurs, there are two possibilities (a) the paratyphoid agglutination may be an instance of group agglutination, or (b) the paratyphoid agglutination may be evidence of paratyphoid fever. The differentiation between these two possibilities is achieved, of course, by recording changes in the agglutinin titer upon a repetition of the test or by absorption tests.

Paratyphoid fever is an infrequent disease and the presence of paratyphoid agglutinins is commonly regarded as a relatively infrequent occurrence in the routine typhoid agglutination test so that, at times, it becomes a matter for decision as to whether it is worth while to conduct all three tests on sera submitted for routine examination.

A survey of this phase of the question has recently been reported by Gilbert and Groesbeck.¹ Of 13,644 sera routinely tested, an average of 0.09 per cent showed complete agglutination of paratyphoid bacilli and 0.7 per cent showed partial agglutination.

As a result of these findings these observers concluded that the test for paratyphoid agglutinins could be omitted from the routine examination except when paratyphoid infection was suspected.

While the clinical course and treatment of typhoid and paratyphoid infections have much in common, their differentiation is desirable in the interests of exact diagnosis and for statistical purposes, and, especially as paratyphoid fever may be overlooked or unsuspected without suggestive leads from the agglutination reaction, it has always been the custom in these laboratories to conduct the three tests simultaneously on each serum examined.

The macroscopic technic using formalinized suspensions, serum dilutions of 1:20 to 1:640 with adequate controls is used. In view of the conclusions of Gilbert and Groesbeck it was thought worth while to survey all the tests made in these laboratories from 1925 to 1927, inclusive, a total of 495.

Of these 188 or 37.9 per cent agglutinated *B. typhosus* the average dilution of the serum in which agglutination occurred being 1:187.

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¹Gilbert R. and Groesbeck W. M. The Occurrence of Paratyphoid Agglutinins in Specimens Submitted For The Typhoid Agglutination Test. *JOUR. LAB. AND CLIN. MED.* 19:5
x No. 4 289

B paratyphosus A was agglutinated by 54 sera or 10.9 per cent, the average titer being 1:156, while B paratyphosus B was agglutinated by 75 sera, or 15.1 per cent, the average agglutination titer being 1:269.

Paratyphoid agglutination was encountered, therefore, in 129 sera or 27 per cent.

All of the positive cases in which clinical disease was present were bacteriologically proved to be typhoid fever and a case of paratyphoid infection was not encountered. As all the paratyphoid agglutinations were group agglutinations, therefore, the strength of the reactions is of some interest.

The lowest agglutination possible within the limits of the test was 1:20 and the highest 1:640.

The varying agglutinin titers for paratyphoid bacilli of the 129 sera in which paratyphoid agglutination occurred are shown in Tables I and II.

TABLE I
PARATYPHOID A AGGLUTINATIONS

SERUM DILUTION	NO POSITIVE SERA	PER CENT
1:20	3	2.3
1:40	6	4.6
1:80	19	16.0
1:160	17	15.0
1:320	4	3.1
1:640	4	3.1

TABLE II
PARATYPHOID B AGGLUTINATIONS

SERUM DILUTION	NO POSITIVE SERA	PER CENT
1:20	2	1.5
1:40	8	6.1
1:80	14	10.0
1:160	18	13.9
1:320	16	12.0
1:640	17	13.0

The relatively high agglutinin titer of these group agglutinations encountered with an unexpected frequency would have led to a suspicion of paratyphoid infection or its presence as a mixed infection were it not for the fact that the clinical course of the disease, the typhoid agglutinin titer, and the bacteriologic examinations of the blood, urine, and feces sufficed to prove the diagnosis of typhoid fever.

In this series, small though it be, the findings of Gilbert and Groesbeck as concerns the infrequency of paratyphoid agglutinins are not confirmed, possibly because the macroscopic technique which is somewhat more delicate, was used as compared to the microscopic method used in their series.

For the present, at least, it will be the custom of these laboratories to test each serum against all strains.

EVIDENCE OF THE SPECIFICITY OF THE INTRACUTANEOUS POLLEN TEST IN MAN*

By R. W. LAMSON, PH D., M D., AND GORDON ALLES, PH D., LOS ANGELES

THE intracutaneous test with pollen extract is frequently used as an aid to diagnosis in cases of asthma, hay fever, or other clinical allergy. It is not employed by certain workers because it is assumed to be nonspecific or at least to fail to distinguish sensitizations to closely related pollens. Several published reports form the basis for such beliefs. Cooke¹ and his associates used this test in a study of grass pollen sensitizations and concluded that "an individual reacting to one reacts to all, which bespeaks a biological identity of the proteins derived from the pollens of the graminaceae." Coca found a single exception to this rule. Brown² agreed with Cooke and extended the previous conclusions in that he believes "that patients reacting to the pollen of short ragweed will usually react also to other members of the compositae." VanderVeer³ and Kahn⁴ apparently agree with the previous views. Others have published observations pointing to an identity of the excitants in low and high ragweed. Walker⁵ in a small series obtained little evidence of identity of any of the so called fall pollens. He reports but one case that reacted equally strongly to all members of the compositae. In view of these theories it seems logical to speculate as to whether these observations are but manifestations of a fundamental biologic law governing all groups of hay fever plants. The observations which follow are submitted as evidence in this connection.

Two important components of the test must be taken into account, though each cannot be studied entirely separate. First, the reactive capacity of the skin may be assumed to be incapable of demonstrating specificity. That is, any protein solution or irritant might, when injected into the skin, produce an urticaria like response, or the material tested might react one day and not the next and such alternation continue without end. If this were the case, of course, no significance could be attached to the test. The second factor is concerned with the reactive constituents in the pollen extract. If these are identical within a family or genus then a test with a single pollen should indicate skin sensitization to pollens of that particular group. Chemical determination of nitrogen partition or attempts to establish any particular chemical constituent as characteristic of a single pollen do not, as yet, yield results that may be identified with species or other botanic subdivision. A recent study⁷ in this laboratory of the micro morphologic characteristics of grass pollens disclosed no significant difference which could be correlated with genera though certain differences were noted among the fall pollens.

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The present study was designed to furnish data relative to the problems indicated above. The first point considered was duplication of the tests, the second, skin reactions to several species within a genus and in one case to two subspecies within a species. Some of the data have been indicated in a preliminary report,⁸ and we now wish to present the complete data.

METHODS

The method of making pollen extracts has been outlined in detail in another article.⁹ The test solution is a 1-1000 extract. Approximately 0.02 cc of this solution is injected into the skin on the lateral aspect of the upper arm. The reactions are read in fifteen to twenty minutes. The method of recording results is based on a numerical estimate of the degree of the reaction. A four-plus reaction is the largest and has definite pseudopods. It would correspond to McLaughlin's¹⁰ Fig. 1. His Fig. 2 we would call a three-plus, the wheal is enlarged but not to the extent seen in the more positive reaction. One or more small pseudopods must be present. All other reactions which show differences from the control are considered doubtful. They are recorded as two-plus, but most of them are undoubtedly negative. The tendency is to overestimate the degree of positiveness. We lean to the side of conservatism but the borderline reaction is always difficult to evaluate. A marked two-plus reaction might actually be a weak three-plus, etc. Sex and age distribution are quite uniform throughout the several groups. These patients are known to have a clinical allergy, asthma and hay fever being the most common diagnoses in the series.

One of the most important criteria in judging any analytical method is the frequency with which duplication of results may be obtained. We have applied this measure to the intracutaneous test and the results obtained are given in Table I.

The extracts of four different pollens were employed in this series of 27 allergic patients. Each pollen was tested twice using solution from two separate bottles, A and B. The tests were placed about one inch apart in the long axis of the arm. The first 10 cases are those giving definitely positive reactions to *Cynodon dactylon* in duplicate and to practically no other pollen. In the last four of these, moderately positive three-plus reactions were obtained to some of the other pollens on one of the duplicates and in most cases the second test was recorded as a two-plus. Cases 11 through 15 gave definite reactions to *Ambrosia psilostachya* in duplicate, and in the last case only was there a mild reaction to another pollen, and the second test of this pair was considered doubtful. Probably these discrepancies in duplicates are instances of borderline reactions. A study of the data presented will indicate exact duplication of the reactions both positive and negative in the same patient in the majority of the cases. Other studies^{9, 11} have shown that duplication of a majority of skin reactions to pollen is possible even when a two-week interval elapsed between tests. Absolute mathematical precision is not claimed for this skin test, but it does appear to have an accuracy comparable to other tests involving biologic factors.

TABLE I
DUPLICATION OF INTRACUTANEOUS TESTS

CASE NO	AMBROSIA PSILOSTACHYA		ARTEMISIA TRIDENTATA		ATRIPLEX POLYCARPA		CYNODON DACTYLON	
	A	B	A	B	A	B	A	B
1	-	-	-	-	-	-	4	4
2	2	-	-	-	-	-	4	4
3	2	2	-	-	-	-	4	4
4	-	-	-	-	2	-	4	4
5	2	2	-	-	-	-	4	4
6	2	2	2	2	-	-	3	3
7	-	2	-	-	3	-	4	4
8	-	-	-	-	2	3	3	3
9	3	2	3	2	-	3	4	4
10	-	2	3	-	2	-	4	4
11	4	3	-	-	-	-	-	-
12	4	4	-	-	-	-	-	-
13	3	3	-	-	-	-	2	-
14	4	4	-	-	2	-	-	-
15	4	4	2	-	3	2	-	-
16	4	4	4	4	-	-	-	-
17	4	3	4	4	-	-	-	-
18	3	3	-	-	-	-	4	4
19	-	-	4	4	-	-	4	4
20	-	-	4	4	-	-	3	3
21	4	3	2	2	4	4	4	4
22	3	3	4	4	4	3	2	-
23	4	4	4	4	4	4	4	3
24	4	4	4	4	4	4	4	4
25	4	4	4	4	4	4	4	4
26	4	4	4	4	4	4	4	4
27	4	4	3	3	4	4	4	3

In Table II a summary is presented of the reactions obtained on 105 patients with pollens from four different genera. The pollen of ragweed reacted in 75 per cent of the cases and Allscale pollen in about 43 per cent. The pollen of Bermuda grass, a common sensitization in this section of the country, reacted in about 50 per cent of the cases.

TABLE II

DISTRIBUTION OF REACTION TO FOUR COMMON FALL POLLENS. TOTAL NUMBER OF PATIENTS OBSERVED, 105, 61 MALES AND 44 FEMALES

POLLEN TESTED	NO. REACTING
Ambrosia psilostachya (Western ragweed)	75 patients
Artemisia tridentata (Sagebrush)	51 "
Atriplex polycarpa (Allscale)	44 "
Cynodon dactylon (Bermuda grass)	55 "

These two tables furnish data tending to refute the claim that an individual skin sensitive to one of the compositae is usually sensitive to other members of this family or that there is necessarily any correlation between the reactive substance in pollens more widely separated than those genera within the compositae.

In Tables III, IV, and V we will attempt to show that the skin reactions to pollens within a genus are not uniformly positive or uniformly negative on the same patient. The individuals considered in Tables III and IV were tested with six species of *Artemisia*, one of these being represented by two subspecies. Twenty-two per cent of those tested reacted to but one of the six pollens, and 60 per cent of them reacted to not more than three (one-half) of the pollens tested. In the subspecies of *Artemisia vulgaris* the botanical differences are but minor ones, yet 24 out of the 50 patients tested reacted to but one of the two subspecies. It will be noted that no pollen consistently gave negative reactions, and that none reacted on all the patients tested. Although the reactive factor was shown to be present in each extract this factor was inactive in the absence of skin sensitization.

In Table V essentially similar results are shown for the seven species of *Atriplex* studied. Over 20 per cent of the patients reacted to but one pollen and 65 per cent reacted to not more than three pollens of the seven.

TABLE III

DISTRIBUTION OF REACTION AMONG SIX SPECIES OF *ARTEMISIA* (SAGEBRUSH)

		SPECIES					
NO OF POL LENS RE ACTED TO	NO OF PATIENTS	BIENNIS	CALIFORNICA	CAMPESTRIS	DRACUNCULUS	TRIDENTATA	VULGARIS*
1	18	4	4	3	1	4	2
2	19	6	2	3	7	10	11
3	12	6	4	7	6	7	6
4	9	5	5	6	5	7	8
5	8	8	5	7	6	7	7
6	16	16	16	16	16	16	16
Totals	82	45	36	42	41	51	50

TABLE IV

DISTRIBUTION OF REACTIONS AMONG SUBSPECIES OF *ARTEMISIA VULGARIS*—SP (MUGWORT)

NO OF PATIENTS	REACTING TO BOTH	REACTING TO UDONICIANA ONLY	REACTING TO HETEROPHYLLA ONLY
50	26	18	6

The data in these tables indicate that an allergic patient does not necessarily react to all "fall" pollens; if he reacts to one, he may react to but one within a genus or to but one of a subspecies. The reaction, therefore, seems capable of differentiating between pollens even though chemical criteria are inadequate. This phenomenon has been demonstrated in a study of reactions to grass pollens¹² wherein it was shown that a definite skin hypersensitive individual might consistently remain negative to one or more common grass pollens, in spite of injection of these pollens and natural exposure to them for one or more years.

In the preceding discussions we have purposely avoided any reference to the clinical significance of the particular skin reactions. Nothing in the data presented could be construed as evidence in favor of or against a direct relationship between the skin reactions and the clinical significance thereof. These

data are presented and analyzed from the standpoint of the skin reactions only and seem to justify us in making certain deductions

TABLE V
DISTRIBUTION OF REACTIONS AMONG SEVEN SPECIES OF *ATRIPLEX* (SCALES)

NO OF POL LENS RE ACTED TO PATIENTS	SPECIES							
	NO OF ARGENTEA	BRACTEOSA	CANESCENS	LENTIFORMIS	PATULA	POLYCARPA	ROSEA	
1	16	4	2	2	3	1	2	2
2	14	1	6	5	5	2	2	7
3	17	10	10	8	5	8	3	5
4	9	5	4	6	3	5	8	5
5	8	2	7	7	3	6	8	7
6	3	3	3	2	3	2	2	3
7	5	5	5	5	5	5	5	5
Totals	72	30	37	35	27	29	32	34

CONCLUSIONS

1 By the employment of the methods outlined, duplication of intracutaneous tests with pollen extracts in the skin hypersensitive individual was possible in a large majority of the cases

2 A strongly positive skin reaction to one pollen in the allergic patient is not necessarily accompanied by a corresponding skin sensitivity to all other pollens. In fact the majority of patients did not react positively to one half of the pollens within a genus. Evidence is submitted to show that a similar phenomenon may be manifested when pollens within a subspecies are tested

3 These findings are considered evidence that the intracutaneous test is specific and *a priori* that the reactive capacity of the patient's skin, and the reacting substance in the pollen extract manifest specificity. The test may be of value in separating closely related pollens even though morphologic studies of the pollen grains or chemical examination of the extract are inconclusive

4 Absolutely no attempt is made to correlate these reactions with the etiology in an allergic patient

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HEMOAGGLUTINATION

1 HEMOAGGLUTINATION IN THE BLOOD OF INFANTS

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BY THIS term is understood that peculiar phenomenon known as the clumping of erythrocytes when mixed with the serum of another animal. If the animals belong to the same general species, it is known as iso agglutination, if they be of different species, it is called hetero-agglutination.

For many years it has been known that blood, withdrawn from one animal and injected into the blood stream of another, will often produce immediate, alarming, and even fatal results. During the past quarter of a century much scientific inquiry has been made to determine the cause and nature of this reaction. Landsteiner, in 1901, found after a study of the blood in a small series of cases, that the fatal results were probably due to agglutination of the red blood cells, he also found that human blood could be divided into three distinct groups. Heektoen in 1907 confirmed these findings, and Jansky in the same year added another group, making four in all, each marked by characteristic reactions. Accordingly, he worked out a table for the classification of human blood based on the phenomenon of iso-agglutination. Some two or three years later, Moss, working independently, framed a table identical with the Jansky classification, except that Groups I and IV are in reverse order. For some reason, other than right of discovery, the Moss system has been most generally adopted. It is the one used in this paper, although full credit belongs to Jansky because of his prior work.

Since 1910 many observers have invaded this interesting serologic field, and separately they have arrived at conclusions more or less contradictory. It is not the intention to contrast those findings or pass upon the claims of the various investigators, except as their reports deal with problems that lie within or adjacent to the province of this paper, namely, the blood of infants.

During the late World War when transfusion became so urgent because of the numerous cases of hemorrhage, quick, easy methods of blood typing were highly desirable, and several simple procedures were evolved. In general, they all fall under two groups, the macroscopic and microscopic. The former method involves the use of serologic tubes carrying mixtures of red

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cells and serum together with controls The agglutination is seen as masses of clumped red cells which sink to the bottom of the tubes While this method is accurate and highly satisfactory in the hands of trained experts it offers no advantage over the quick and simple microscopic method for the average typist The latter method, therefore has been followed exclusively

Technic employed

1 Moss' Classification —

SERA TYPES

CELL GROUPS	1	2	3	4	PER CENT
I	0	+	+	+	8
II	0	0	+	+	40
III	0	+	0	+	10
IV	0	0	0	0	42

Explanation of Table

Group I The serum of Type I does not agglutinate the red cells of any of the four groups of cells the corpuscles of that group however, are clumped by the sera of the other three types

Group II Type II gives a positive reaction with Groups I and III negative with II and IV

Group III The Type III serum will agglutinate the red cells of Groups I and II, it will not clump those of Groups III and IV

Group IV While none of the four types of sera will agglutinate Group IV red cells its own serum will clump those of the three other groups

2 Vincent's Open Slide Agglutination Method — This method was followed exclusively because of its accuracy and simplicity It consists of the following procedure

T 2 S	T 3 S
0	0
R C X	R C X

a Place one drop of No 2 serum on the left end of the slide Place one drop of No 3 serum on the right end of the slide

b Mix one small loopful of prepared red cell suspension with each drop of serum, respectively, taking care to flame the loop before each mixing

c Red cell suspension is prepared by centrifuging the citrated blood decanting off the supernatant fluid then washing the cells in normal salt solution Since the umbilical blood was collected in sterile normal salt solution containing 1 per cent sodium citrate, this portion of the procedure was found quite unnecessary accordingly, loopsfull of red cells were taken directly from the vials From making many tests no difference could be detected in changed reactions or in the amount of time required for agglutination therefore this portion of the technic was omitted

d At regular intervals of two or three minutes the slide should be agitated by alternate tilting to aid diffusion and hasten the process of clump

ing This is usually complete within ten or fifteen minutes Cover-slips are not necessary if examined at once The microscope should be used in every case

3 *Stock Sera*—Inactivated stock sera, Types II and III, adult, were secured from the Virginia Mason Laboratory and preserved in sealed, capillary tubes in the ice box No deterioration in potency was detected before each quantity of stock sera was replenished, some two months after withdrawal No preservative was used, and no special preparation required other than above mentioned

4 *Umbilical Blood*—This was obtained from various private hospitals through cooperation of obstetric departments and the attendant physicians After delivery the umbilical cord was cut and drained into twin-batteries of two-dram vials, each, before expression One empty vial received blood which was allowed to clot and the serum later removed as needed, the other vial, partly filled with 1 per cent sodium citrate in normal salt solution, received blood which did not clot These specimens were preserved in an electric refrigerator and examined as soon as possible, usually within forty-eight hours after collection Frequently, a week or so after the discovery of a good, strong reacting umbilical serum, its potency on retest with both the original and new red cells was well preserved So far as determined, if both the red cells and serum were stored in the refrigerator and preserved from bacterial invasion, agglutination persisted If the specimen was grossly contaminated, the clumping phenomenon was soon destroyed

It should be noted here that the specimens of umbilical blood were secured under the most favorable conditions The vials containing the citrate solution were twice autoclaved, once when prepared and again before going to the obstetric wards, to prevent contamination of the delivery room The blood from the cord was then drawn into these sterile containers and, if not later contaminated, was preserved for a considerable period of time

TABLE I
RESULTS OF TYPING UMBILICAL RED CELLS

TYPE	SEX	CASES	TOTAL CASES	TOTAL PER CENT
I	M	2	6	7 1/2
	F	4		
II	M	15	32	40
	F	17		
III	M	3	8	10
	F	5		
IV	M	18	34	42 1/2
	F	16		
Total	M	38	80	100
	F	42		

Explanation of Table I—As stated before, the red cells were tested with fresh human serum of known types, II and III, for group determination It will be seen that the percentages found for the four different general types

of umbilical blood correspond quite closely with the percentages usually allotted for adult blood. In other words, the agglutino-genic property of erythrocytes appears fully developed and quite normal at birth. There existed no opportunity to recheck the infant's blood at a later period to learn if the postpartum type persisted; neither was it possible to compare the fetal types with those of maternal blood.

Of the eighty cases examined, the sexes are represented about equally, thirty eight males and forty two females. Little difference could be found in this series of typings from that standpoint except perhaps, a slightly higher percentage of the less common groups for girls.

TABLE II
RESULTS OF CROSS AGGLOUTINATING UMBILICAL CELLS WITH UMBILICAL SERUM

SERA X		SERUM Y	
O		O	
R.C.Y.		R.C.X	
	NO. CASES		PER CENT
Both positive	8		11 2/3
One positive	24		35 1/3
Neither positive	36		53
Total cross agg.	68		100

Explanation of Table II.—This shows a series of sixty eight cases of cross agglutinations, e.g., umbilical serum X, was mixed with umbilical red cells Y, while the blood serum of Y was typed with the red cells of X, respectively on opposite ends of a microscopic slide, as shown in the table heading. Agglutination occurred in only eight cases simultaneously, at both ends of the slide, in twenty four cases a single test showed positive while in thirty six trials neither mixture showed agglutination. It will be further noted that the typings were made in pairs, so that the sixty eight cases represent, in reality one hundred thirty six trials. According to the Moss classification Type I serum will agglutinate neither of the four groups of cells, nor are the cells of Group IV clumped by any of the normal types of sera. It follows therefore that combinations of sera and cells made at random will include many negative tests. Of the combinations tabulated above, i.e., the one hundred thirty six trial agglutinations, seventy one were theoretically positive but only forty showed positive by actual test. In other words, of the theoretically positive reactions only 56 per cent showed the clumping phenomenon. In some cases these reactions were vigorous and appeared promptly others proved weak and somewhat delayed, still others, which should have occurred, did not appear. It is therefore quite apparent that the agglutinin of umbilical serum does not show the same degree of reactivity as shown by the serum of an adult.

In Tables I and II, the findings confirm in a general way the observations of De Biasi, reported in 1923. He found in a series of one hundred cases of umbilical blood, the following group percentages, according to the Moss classification:

Group I	12 per cent
Group II	24 per cent
Group III	17 per cent
Group IV	47 per cent

He also found that it was possible to arrange the red cells according to this classification scheme. While his percentages differ slightly from these findings, as shown in Table I, nevertheless they are as a whole, confirmed. Therefore, his statement may be concurred in that "corpuscles of the newborn infants have their quota of receptors."

As regards his findings in infant serums, in no cases were the maternal red cells agglutinated by the serum of the infant, in four cases only did he find that the infants red cells were clumped by maternal serum. This result one would naturally anticipate, since the red cells of the child are developed from its own tissues, whereas there must necessarily exist some osmotic exchange between maternal and fetal sera, in other words, the serum of the newborn is a mixture. By cross-typing one umbilical blood with another, agglutinin if present and well developed in the serum of the newborn should give the same reactions and percentages shown by normal adult sera when typed against these cells. This is not true, as shown in Table II. In only about one-half the cases are theoretically positive agglutinations confirmed by laboratory tests.

CONCLUSIONS

I The erythrocytes of umbilical blood, react in a normal way, and give the same group percentages shown by adults, their agglutinophilic power, therefore, appears fully developed at time of birth.

II Umbilical serum shows in a positive way, its theoretic reaction in about 50 per cent of cases, many of these when present are weak with action somewhat delayed. It appears, therefore, that its agglutinin is often immature or not fully developed at time of birth.

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LABORATORY METHODS

A BLOOD STAIN GIVING MORE CONSTANT RESULTS*

A NEW DEPARTURE IN STAINING WITH ROMANOWSKY STAINS WHICH ELIMINATES
A NUMBER OF SOURCES OF ERROR

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DIFFICULTIES WITH WRIGHT'S STAIN

MOST everyone has had difficulties at times getting satisfactory results with the present methods of staining with the various modifications of Romanowsky stains. Sometimes a specimen of Wright's stain will fail to stain properly and has to be discarded, whereas the next sample, made up apparently in the same way and from the same ingredients, will prove quite satisfactory.

At other times the difficulties may arise from failing to remember the staining time or the proper amount of water needed to dilute the stain, or from accidental bungling in handling the slide during the process of staining. As a result some smears are not stained deeply enough for use while others are heavily stained and perhaps covered with a dense film of precipitate which obscures all details. The beginner particularly is prone to have difficulty in obtaining well stained smears.

Often these failures are discouraging because it is difficult to locate definitely the factor causing the poor results. On several occasions we have happened to have poor luck just when we had a set of particularly interesting slides which we wished to stain for class work or for demonstration purposes. We have also observed in looking over loan collections in other departments and other schools, that blood smears are often so poorly stained that they fail to show the things which they are supposed to illustrate.

For these reasons we undertook some time ago to investigate the factors responsible for failures in getting proper results with Wright's stain, hoping at the same time to hit upon a method which would give more constant results.

SEARCH FOR A DECOLORIZING SOLUTION

Since overstaining and precipitated stain seemed so often to be the crux of the difficulty, it seemed to us that one way out would be to purposely overstain the smears and then partially decolorize them. We had already made use of the expedient at times of removing precipitate with ethyl alcohol. However, it was quite easy to completely decolorize the smear in this way, the nuclear blues being particularly attacked, so some slower acting and more differential decolorizing solution had to be found.

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Experimentation with all of the other common solvents used in the laboratory proved fruitless. They removed either all or none of the stain. Mixtures of these solvents in varying proportions likewise proved useless.

It occurred to us that something might be added to the ethyl alcohol to slow its action or make it more differential. Since the blues were especially attacked by this solvent, we added varying quantities of methylene blue, finding that a saturated solution gave fair results, but much of the azure was lost. This fact suggested the use of Wright's stain, itself, in the decolorizing solution, as the azures might then be retained. The results obtained with this solution, after learning to adjust the factors, as described below, which cause variations in the stains, were very satisfactory.

PREPARATION OF SOLUTIONS

Solution I—This consists of Wright's stain dissolved in absolute methyl alcohol in the usual way. However, we prefer to prepare the solution by placing enough of the dry powder into a dry clean bottle to saturate the amount of alcohol added (not more than about 0.3 gm. will dissolve in 100 cc.). The solution should be allowed to stand for several hours, preferably a day or two, with occasional shaking. It should then be filtered and diluted with one-fifth of its volume of methyl alcohol.

Solution II—This consists of a saturated solution of Wright's stain in 85 per cent to 90 per cent ethyl alcohol (only about 0.2 gm. will dissolve in 100 cc.). We usually prepare this solution by using nine parts of 95 per cent alcohol to one part of distilled water as the solvent. This solution must stand for not less than twenty-four hours, preferably longer, with occasional shaking before it can be used, because saturation is slowly reached when the excess of stain present is so small. It is well to filter before using, otherwise some of the undissolved stain is likely to be poured on the slide and fail to be washed off.

DIRECTIONS FOR USING STAIN

1. Drop on just sufficient stain (Solution I) to cover blood smear, *drain off excess stain* at once, let stand until all of the stain remaining on the slide turns red.

2. Flood with distilled water and allow to stand for one or two minutes.

3. Wash with decolorizing solution (Solution II) until most of the red precipitate disappears. That over the smear usually disappears immediately, and any at the ends of the slide may be wiped off with a cloth.

4. Wash with distilled water, dry and examine. *Note* Drying at any stage *after the excess stain is drained off* does not materially harm the preparation.

FACTORS CAUSING VARIATIONS IN STAINING

During this study we have found at least four important factors which influence the character of colors obtained. Thus greater intensity of red is obtained by (1) A more acid water, (2) a higher percentage alcohol in preparing Solution II, (3) a more concentrated Solution I, (4) a longer staining time in the presence of water. Conversely, a less intense red is produced

by the reverse of these factors. The four are to a certain extent interdependent, a change in one being compensated for by a change in another.

We have studied somewhat in detail the effect of the P_H upon the colors obtained. Using water buffered with phosphate solutions, we find that the colors which we prefer are obtained when the water is a P_H of 6.4 to 6.8. The colors are fairly satisfactory for differential counting with a P_H as low as 5.6, but the red cells are very pink and the nuclear blues of the leucocytes hazy and indistinct. On the other hand, when the P_H goes beyond 7.4 the red cells take on a greenish blue hue and the granules and nuclei of the leucocytes fail to take the eosin and azures properly.

Distilled water is usually slightly acid and consequently makes a satisfactory diluting and washing medium without buffering. However, the amount of acid is subject to variation, especially where the water is distilled from city supplies which are chemically treated. For this reason we prefer to use a water which is buffered.

A simple method of preparing such a water is to keep on hand 1 per cent solutions of KH_2PO_4 and Na_2HPO_4 . Ten cc. of the latter are used with each liter of distilled water, and the KH_2PO_4 is added in 1 to 5 cc. quantities until the desired color of red cells is obtained when smears are stained. The quantity usually needed is 10 to 30 cc., depending upon the personal preference as to shade. We like the color to be a slightly pinkish buff.

In regard to the second factor, the best strength of ethyl alcohol to use in preparing Solution II is 85 to 90 per cent. Stronger alcohol often fails to remove the red precipitate left in overstaining the smear, and weaker alcohols remove both reds and blues, even to complete decolorization.

As to the third factor, it often happens that a stain which sits in a dropping bottle for a long period loses alcohol by evaporation and becoming concentrated causes overstaining with eosin. Usually diluting again with methyl alcohol restores the proper balance of staining.

PIGMENTS USED WITH THIS PROCEDURE

Most of our experimentation has been done with Wright's stain produced by American manufacturers. All samples which we have tried have given satisfactory results. It seemed well, however, to discover if pigments made in other ways would be equally successful. For this reason we have prepared pigments by all of the processes usually employed in polychroming methylene blue and making the eosinates.

Several specimens have been prepared by Wright's, Leishman's, and Wilson's methods, and all were satisfactory. We have also procured samples of the latter, two from manufacturers, and tried them out with equal success. Personally we like the qualities of the pigment produced by Wilson's method as described by Svehla¹ better than any of the others.

It is interesting to note that some of the samples used worked poorly when the usual staining method was employed, but gave beautiful stains with the method which we have devised.

ALCOHOLS USED IN PREPARING SOLUTIONS

In order to obtain good results with Wright's stain by the usual procedures it is generally advised to use a special absolutely pure, acetone free methyl alcohol. With this procedure we have been able to secure good results even with commercial wood alcohol, the principal disadvantages being that some samples contain impurities which are likely to leave more precipitate on the smear, and some are not near enough 99 per cent purity to properly fix the cells in the thick portions of the smear. However, this does not interfere materially with recognizing leucocytes or blood parasites, and for routine work, where the slides are discarded after examination, such alcohols are often satisfactory enough.

We have also found that the commercial denatured alcohols can be used in making Solution II, especially if wood alcohol is the principal material used in denaturing. Of course, there are a large number of formulas in use in denaturing, and no doubt some substances used in the process might render the sample unfit for this purpose.

We have procured samples of these two alcohols from a number of sources, drug stores, wholesale dealers, etc., and have used specimens on the shelves of various departmental laboratories, and none which have been refined enough to be perfectly clear and colorless have failed to give success.

CHARACTERISTICS OF STAINS OBTAINED

We have stained hundreds of slides by this procedure with very uniform results and practically no failures. It has worked satisfactorily in the hands of a number of students who have tried it out parallel with their Wright's stain. Likewise, a number of our personal friends in other medical schools have been kind enough to try out samples and have reported results comparable to our own.

The differentiation between the leucocytes is sharp. There is no massing of stain upon the leucocytes to mask internal structures, and the granules stand out plainly. The granules of the neutrophils are quite delicate but discrete, those of the eosinophiles a definite red, and those of the basophiles a very deep blackish purple. Likewise, the azurophilic granules of the lymphocytes are very definitely demonstrated, even in smaller cells.

The red cells may be stained any color desired from a light yellowish buff to a very distinct pink, depending upon the proper adjustment of the factors mentioned above. Due to the delicate coloring of the erythrocytes, polychromatophilia and basophilic stippling are clearly demonstrated.

Fortunately, during the period of our experimenting, we have been able to obtain large numbers of smears from cases of leucemia, and of tertian and quartan malaria, and much of our staining has been measured by how satisfactorily the malarial parasites and the granules of the myelocytes were brought out.

SUMMARY

1. A method of overstaining with Wright's stain, and partially decolorizing, is described.

2 Factors causing variations and failures in staining, and their proper adjustment, are discussed

3 Commercial alcohols can be used in routine work

4 Constant satisfactory results are obtained by those who have used this method

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RAPID AND ROUTINE PREPARATION OF TISSUE SECTIONS*

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DESPITE the fact that numerous technics for the rapid and routine preparation of tissue sections have been devised during the past ten years, many of the methods at present in use, while producing reasonably good routine sections, are too time consuming for the diagnosis of cases in which a radical surgical procedure is anticipated should the tissue removed prove to be malignant. The freezing method with polychrome methylene blue staining answers the purpose extremely well when a definitely benign or malignant tissue is found, in as far as rapidity is concerned, but where a borderline tissue is to be pronounced definitely malicious or nonrecurrent, and a major operative procedure is to be undertaken or otherwise depending on the pathologist's interpretation of the sections a grave feeling of responsibility and doubt of one's reading must necessarily ensue as he studies a section distorted by freezing and not showing the best detail possible as the result of poor polychrome stain infiltration.

The following method has been used by me for the past six years, and while consuming about six hours from the time the tissue is received until the time the surgeon has his report, the method is sufficiently fast for all practical purposes, at the same time producing permanent sections which it is a pleasure to interpret. The cells show no distortion or shrinkage, are free from artefacts, and show very definite clear cut nuclear and cytoplasmic stain detail. In addition to these facts the method is applicable to routine as well as urgent work. My laboratory has been in the habit of using the technic for all tissues, in this way cleaning up all the pathologic specimens the day after they are received as far as the surgeon is concerned, unless for some reason special staining methods are deemed advisable.

Pieces of tissue of sufficient area to facilitate an adequate study of the specimen are cut as thin as is convenient to handle and should in no case be over 2 mm thick for the rapid method. The cutting surface may be of any size up to one by three inches, though three quarters of an inch square or smaller has been found to be a convenient size for routine work. In case

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thicker pieces of tissue are desired, as for serial sections, they must necessarily remain longer in the fixing reagents and are not applicable to the rapid method. Also they are more subject to shrinkage from remaining in the fixatives longer. The tissues are threaded on a length of fine sewing cotton with the aid of a fine needle. A small glazed paper or parchment tag is threaded on, after all the sections from one source, bearing an identification number in ink. In this way many sections or sets of sections may be placed on one thread. Uterine curettings are massed loosely and tied in a single layer of gauze before being placed on the thread.

Eight one-quart covered jars are required for the fixing reagents and two small enameled pitchers for the paraffin. Ordinary fruit jars with clamp tops are ideal for the reagents. The first jar is nearly filled with 85 per cent alcohol, the second with 90 per cent alcohol, and the third with 95 per cent alcohol. Acetone is placed in the fourth jar, oil of cedar leaves in the fifth, chloroform in the sixth and seventh, and a saturated solution of paraffin in chloroform in the eighth. The jars should be kept nearly full of reagent. One worker I saw using the technique was actually trying to get results with half an inch of oil of cedar leaves in the jar when he was running through six to eight tissues each day. As the alcohol becomes hydrated only the first needs to be discarded, the second replacing the first, the third the second, and only the third needing to be replaced by new. In time the first chloroform will become quite yellow from oil of cedar leaves and will need discarding. The other reagents will last almost indefinitely, needing only to have their volume replenished from time to time. This brings about an element of economy which is usually appreciated where large numbers of tissues are run through routinely. The two pitchers are numbered one and two and contain paraffin of about melting point 55°C , and are kept in the paraffin oven, which should be just hot enough to keep the paraffin liquid but should never be over 58°C or the tissues will tend to scorch unless very closely watched.

The fresh threaded tissues are placed in the first alcohol for half an hour, transferred to the second for a similar period, and are then allowed to remain in the third for two hours. They are then run through the other reagents remaining fifteen minutes in each. No preliminary formalin or other fixation is necessary, the only advantage to be gained by a preliminary fixation being a tendency to distortion from shrinkage. Formalin or Zenker's fixed tissues run through this method stain equally as well as those not so treated. After fixation the tissues are placed in first one paraffin and then the other, remaining fifteen minutes in each.

While the tissues are in the oven, small paper boxes may be folded from any stiff paper large enough to accommodate one or more sections. These are filled with paraffin and the tissues unthreaded and immersed while the paraffin on them is still molten. The identification numbers are written on the ends of the boxes, and the boxes placed on the ice in the refrigerator to harden, a process which should take not more than twenty to thirty minutes at the most to obtain good hard blocks.

The paper is then removed and the blocks trimmed. Trimming is an important step if long ribbons of serial sections are desired. Two sides of the block should be trimmed fairly close to the tissue, and the other two, which will form the top and bottom edges when the block is in the microtome, should have a paraffin margin of approximately 3 mm. Good sections cannot be expected if the microtome knife is not sharp or has nicks in it.

Sections may be stained by any method desired, the following giving uniformly good results. After mounting of the tissues on glycerine albumen smeared slides they are set on edge in the paraffin oven until the paraffin has melted, usually five to ten minutes. The slides are then cooled either in the refrigerator or at room temperature just long enough for the paraffin to crystallize and are then run through the following stain process:

- 1 Xylol 1 for five minutes
- 2 Wash by gently swishing back and forth in xylol 2 and 3, 95 per cent alcohol, and water for thirty seconds each
- 3 Delafeld's hematoxylin fifteen minutes
- 4 Rinse in water
- 5 Decolorize in 1 per cent HCl in 70 per cent alcohol by dipping in slowly two or three times
- 6 Wash in running water until blue twenty minutes
- 7 Counterstain in $\frac{1}{2}$ per cent aqueous eosin three to five minutes
- 8 Rinse in alcohols 1, 2, and 3
- 9 Clear in 0.3 per cent carbolic acid in xylol three minutes
- 10 Rinse in xylol 4 and 5 and leave in this until ready to mount
- 11 Clean any undesired tissue off of the slide with a clean towel without letting the section become dry. Place a drop of xylol balsam on the center of the section and press a cover slip down on it.

This staining technic may be speeded up by leaving the slide in hematoxylin only five minutes, rinsing only momentarily in acid alcohol and using very dilute ammonia water to develop the blue color instead of leaving in running water for twenty minutes.

In routine work the tissues may be kept in air tight jars until the afternoon, the gross examination then performed and the tissues run through as far as the third alcohol remaining in this until the following morning.

Tissues cut and stained according to this technic should be free from artefacts, should cut and stain easily whether large or small, show good differentiation whether for microscopic study or photomicrographs and should give as true a histologic picture as is at present possible to obtain.

A COMPARATIVE STUDY OF THE EFFICIENCY OF DEHYDRATED ENDO'S AGAR AND KRUMWIEDE'S TRIPLE-SUGAR AGAR*

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DEHYDRATED media of various kinds have been placed on the market by certain of the commercial laboratories. Such media, if they proved to be as efficient as media which had not been dehydrated, would be of great value in many small laboratories. These media which are prepared in the laboratory by dissolving the dry material in distilled water, dispensing, and sterilizing, would have the advantage not only of saving time and material but also of tending to make results obtained in one laboratory more comparable with those obtained in another through the use of more nearly uniform media.

This study was undertaken to compare the efficiency of dehydrated Endo's agar and Krumwiede's triple-sugar agar made by one of the commercial houses, with the corresponding standard media¹ used in this laboratory in the routine examination of specimens for organisms of the enteric-disease group. At the time this work was done, the standard Endo agar in routine use in this laboratory was made according to a modification of the formula of Robinson and Rettger,² and the triple-sugar medium was a modified³ Russell's⁴ double-sugar medium.

The efficiency of the two Endo agars was compared by determining how well and typically strains of the colon-typhoid group of organisms grew on each of these media, and by the extent to which organisms found in fecal specimens containing incitants of enteric diseases could be differentiated from one another when grown on each of these agars. On the triple-sugar media, comparison was made of the reactions produced by pure cultures of various organisms. These media were further studied by observing the reactions produced in each by the growth of a single colony fished from Endo's agar inoculated with mixtures of *B. coli* and *B. typhosus* or *B. dysenteriae*, or with routine specimens containing organisms of the enteric-disease group.

TECHNIC

The cultures used were *Staphylococcus aureus*, *B. coli*, *B. typhosus* Bender strain, *B. paratyphosus* A, *B. paratyphosus* B, and three strains of *B. dysenteriae*, Flexner, Mt. Desert, and Shiga. The Endo-agar plates were inoculated with suspensions of these cultures made by emulsifying in 0.85 per cent salt solution the growth from agar slants which had been incubated for eighteen hours at 37° C. The tubes of triple-sugar media were inoculated directly from the eighteen hour agar slant cultures.

The mixtures of *B. typhosus* and *B. coli* (1:5, 1:10, 1:15, 1:20) and of *B. dysenteriae* Mt. Desert and *B. coli* (1:6, 1:12, 1:24, 1:30, 1:36) were prepared from eighteen hour broth cultures of the same density and diluted to very light suspensions with sterile broth.

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The specimens with which the Endo agar media were tested were suspensions of feces in glycerin salt solution. All specimens used had already been found to contain *B. typhosus*, *B. paratyphosus*, or *B. dysenteriae*.

Several samples of both the dehydrated Endo agar and the dehydrated triple sugar agar, were obtained from time to time and tested. The composition of two of the samples of dehydrated Endo's medium differed from a third sample both in kind and amount of the ingredients present and in the amount of dehydrated material required to make a liter of medium.

Sample 1		Samples 2 and 3	
Peptone	50 parts	Dipotassium phosphate	3.5 grams
Beef extract	30 parts	Peptone	10.0 grams
Lactose	100 parts	Lactose	10.0 grams
Agar	150 parts	Agar	15.0 grams
Basic fuchsin	0.1 part	Basic fuchsin	0.5 gram
Sodium sulphite	qs	Sodium sulphite	2.5 grams
Dissolve 34 grams in one liter of distilled water		Dissolve 41.5 grams in one liter of distilled water	

The samples of dehydrated triple sugar medium differed in composition only as to indicator. The last sample contained phenol red instead of Andrade's indicator.

Throughout the work with the Endo media, freshly poured plates of the medium made according to the modification of Robison and Rettger's formula were used. The dehydrated medium was made exactly according to the directions on the container and inoculated and incubated on the same day. All tests were carried out in duplicate. Two plates of each medium were inoculated in the following way. One loopful of inoculum was spread over the agar surface of the first plate with a sterile glass rod which was then used to inoculate the second plate. The two plates of the second medium were inoculated in a similar manner. The plates were then incubated for eighteen hours at 37° C. This same procedure was followed with saline suspensions of pure cultures, with mixtures of cultures, and with feces.

The dehydrated triple sugar medium was made sterilized, and inoculated on the same day. Tubes of this medium and of the triple-sugar medium in routine use in this laboratory were inoculated with a straight needle both deep into the butt and on the slant surface. The incubation period was eighteen hours.

When mixtures of two cultures or feces containing organisms of the enteric disease group were plated on the Endo media, colonies which had the characteristic appearance of typhoid or dysentery bacilli were fished to triple sugar agar media. A tube of each triple-sugar medium was inoculated from the same colony and these tubes were incubated together. Whenever the reaction produced in the dehydrated medium did not agree with that of the standard triple sugar agar, other tubes of the latter were inoculated with the culture from the dehydrated triple-sugar medium. If these second tubes of routine medium did not agree with the first, the purity of the culture was investigated by means of Gram stain preparations. One characteristic fishing from each of the Endo media inoculated with specimens of feces containing enteric disease organisms was tested by macroscopic agglutination.

DISCUSSION

The uninoculated dehydrated Endo medium differed in appearance and in hydrogen ion concentration from the Endo agar made according to the standard method. The latter medium was colorless and clear. The plates made from the sample of dehydrated medium which contained 0.1 part of basic fuchsin were clear and slightly colored by fuchsin. The plates made from the samples which contained 0.5 gram of basic fuchsin were deeper in

color and contained a heavy precipitate which was dispersed finely throughout when the medium was shaken just before pouring the plates. The hydrogen-ion concentrations of the dehydrated medium varied from P_H 7.4 to P_H 7.6 while that of the medium made in this laboratory was approximately P_H 7.8.

When plates made with the Endo media were inoculated with saline suspensions of *B. coli*, all plates developed large colonies with pink irregular edges and centers which varied in color from light red on the routine Endo agar and on the sample of dehydrated medium containing the smaller amount of basic fuchsin, to deep metallic red on plates of the samples of dehydrated medium which contained 0.5 gram of basic fuchsin. In this last medium the areas of the plates adjacent to the *B. coli* colonies were deeply colored. This diffusion was less marked with the other sample of dehydrated material and still less apparent in the standard Endo agar.

Staphylococcus aureus grew in small, regular, pink colonies on the dehydrated medium containing 0.1 part basic fuchsin and on the standard Endo agar. However, it was entirely inhibited on plates of the dehydrated medium which contained the higher percentage of fuchsin.

These same samples of dehydrated material also greatly inhibited the growth of the Shiga strain of *B. dysenteriae*. Plates inoculated with this culture developed such very fine colonies after an incubation period of eighteen hours that identification was difficult. If these plates were incubated for a longer period, they deepened so much in color that they were of little use in differentiation. However, plates of the routine medium and of the other sample of dehydrated medium which had been inoculated with the same amount of Shiga culture developed many small characteristic colonies in eighteen hours at 37° C.

The cultures of *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B, and *B. dysenteriae*, except the Shiga strain, grew about equally well on all samples of the dehydrated medium and on the standard Endo agar. The only noticeable difference between the colonies on the various media was their color in reflected light. On the dehydrated medium all typhoid and dysentery colonies were slightly pink, while on the routine medium they were colorless. However, all these colonies were colorless to transmitted light regardless of the medium.

When mixtures of *B. typhosus* and *B. coli* (1:5, 1:10, 1:15, 1:20) and of *B. dysenteriae* Mt. Desert strain and *B. coli* (1:6, 1:12, 1:24, 1:30, 1:36) were plated on the two Endo media, the importance of the difference in the amount of diffusion of the dye was plainly shown. If the mixtures were highly diluted so that very few organisms were present in each loopful of suspension, *B. typhosus* and *B. dysenteriae* could be isolated from all mixtures. With mixtures less highly diluted the color produced by the *B. coli* diffused throughout the entire plate of the dehydrated medium, and the typhoid and dysentery colonies were not apparent in the mixtures containing large numbers of *B. coli*. This was not true of the standard medium where the diffusion was less marked.

Organisms of the enteric disease group were isolated from all of the thirty specimens of feces which were plated on both of the Endo agars. None of these specimens contained large numbers of *B. coli*, probably due to the fact that they had been kept in glycerin salt solution. One positive fishing from each medium was tested by macroscopic agglutination. Variations in amount of agglutination occurred between the cultures grown on the different media, but these variations were not sufficiently marked to warrant any definite conclusion.

The dehydrated triple sugar medium was first tested by comparing the reactions produced in eighteen hours by the growth of pure cultures in the dehydrated medium with the reaction produced by the same cultures in the standard medium. Table I gives the results of several such tests.

TABLE I

COMPARISON OF THE REACTIONS PRODUCED IN THE TWO TRIPLE-SUGAR MEDIA AFTER EIGHTEEN HOURS AT 37

CULTURE	DEHYDRATED TRIPLE SUGAR	STANDARD TRIPLE SUGAR
<i>Staphylococcus aureus</i>	Acid slant	±
<i>B. coli</i>	+g	±g
<i>B. typhosus</i> Bender	±	+
<i>B. paratyphosus</i> A	±g	+g
<i>B. paratyphosus</i> B	±g	+g
<i>B. dysenteriae</i> Flexner	+	+
<i>B. dysenteriae</i> Mt. Desert	±	+
<i>B. dysenteriae</i> Shiga	±	+
	or acid along slant or line of inoculation in butt	
+ = acid in butt only, no gas +g = acid and gas in butt ± = acid in butt and slant, no gas ±g = acid and gas in butt, acid slant.		

All of the samples of the dehydrated medium gave the same results except the one containing phenol red indicator. In this medium *B. dysenteriae* Flexner produced an acid reaction in the slant as well as the butt and *B. paratyphosus* B was the only culture to produce a characteristic triple sugar reaction. The growth of all cultures on all samples of dehydrated triple sugar was poor. The reactions produced by freshly isolated cultures did not differ from those produced by the test cultures. A longer incubation period, from forty to sixty six hours increased the number of characteristic reactions of some of the cultures but such an incubation period would be a great disadvantage even if the medium were entirely satisfactory otherwise.

The only differences in the compositions of the two triple sugar media lay in the amount of beef extract and dextrose present. The standard medium contained 0.05 per cent dextrose and 0.3 per cent beef extract, while the dehydrated medium contained twice as much dextrose and no beef extract. By making small amounts of medium according to the formula used here, but varying the amounts of these two ingredients to correspond with the dehydrated medium, it was found that the test cultures produced many of the same reactions in a medium containing 0.1 per cent dextrose as in the dehy-

drated medium, and that the addition of beef extract had little effect except on the amount of growth produced. However, when 0.3 per cent beef extract was added to the dehydrated medium, not only was the growth improved, but many of the reactions produced by the test cultures became characteristic.

CONCLUSIONS

The dehydrated Endo agar medium though less efficient for the isolation of organisms of the enteric-disease group than the standard Endo agar made according to the Robinson and Rettger modification, supports a good growth of all organisms except the Shiga strain of *B. dysenteriae*.

The dehydrated medium permits a larger amount of diffusion of the dye in the areas of the medium surrounding *B. coli* colonies than does the standard Endo agar. Of the various samples of the dehydrated medium the ones containing the larger amount of basic fuchsin show the more marked diffusion.

When positive specimens preserved in glycerin-salt solution are plated on the dehydrated medium, the amount of diffusion is not sufficient to obscure the typhoid or dysentery colonies.

The results of the comparison of the dehydrated Endo medium with the standard Endo medium indicate that the dehydrated medium while less efficient is nevertheless a usable medium for the isolation of organisms of the enteric disease group with the exception of the Shiga strain of *B. dysenteriae*.

The dehydrated Krumwiede triple-sugar agar is not usable for the isolation and identification of organisms of this group. This medium appears to contain too high a percentage of dextrose and too little nutriment to produce a good growth and characteristic reactions when inoculated with organisms of the enteric-disease group.

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OXYTOMIC APPARATUS WITH TWELVE TUBES*

By E P BUGBEE, M D, AND A E SIMOND, B S, DETROIT, MICHIGAN

IN THE course of the work which led up to the separation of two active principles from the posterior lobe of the pituitary gland¹ several hundred oxytomic tests had to be made. An oxytomic apparatus on which two uterine muscles could be worked simultaneously, was not adequate to handle the great number of tests involved so another apparatus was arranged to work six muscles at a time. Even this apparatus was unable to handle the volume of tests and a new apparatus has been built which can work twelve uterine muscles simultaneously. It employs the same glass muscle tubes and air tubes as have been used for five years by Mr L W Rowe of this laboratory. These in turn are a development from the earlier models described by Hamilton and Rowe in 1916². The six tube and the twelve tube oxytomic apparatus were built for us by Eherbach and Son Company of Ann Arbor, Michigan. Both apparatus have been in use for several months and are very satisfactory. The twelve tube or "twin six" is shown in Fig 1. An electrically heated water bath maintains a temperature of 38° C. Partially submerged in the water stand twelve vertical muscle tubes in which the uterine muscles are suspended. Above the water bath extends a bar which supports twelve upright rods. To the upright rods are clamped the writing levers which magnify the contractions of the muscles and record these contractions on the smoked paper of the kymograph.

The water bath itself is a copper tank 37 inches long, 14 inches wide and 8 inches deep. In the front of the tank are two glass windows, 11½ inches long and 5¾ inches high. The back of the tank is lined with opal glass to act as a reflector as well as to improve the appearance. The tank is covered with insulating material to reduce loss of heat. The tank rests on a base plate of asbestos board supported by an angle iron frame and legs 4 inches long. Flush with the surface of the asbestos board are three electric heating units such as are employed in electric grills. The three heating units are alike and together carry 460 watts. The center unit carries current continuously but the two end units are intermittent in their action. They are controlled by Eherbach thermostatic regulators of the bimetallic type. In parallel with each thermostat there is a condenser to prevent arcing and an 8 CP 250 V carbon lamp to act as a pilot light. The lamp is lighted when the current is not passing through the heating unit. The regulation is entirely satisfactory and the temperature of the water is maintained all day long with a fluctuation of only half a degree. The water tank holds fifty liters of water and the change in temperature of such a large volume of water takes place only very gradually.

From the Medical Research Laboratory Parke Davis and Company Detroit Michigan
Received for publication February 11 1928

The water tank has 13 holes or tubulatures in the bottom. These are of the proper size to accommodate No 6 rubber stoppers. One hole carries the drain pipe for emptying the tank and the other twelve holes carry the drain tubes for emptying the vertical tubes in which the muscles are suspended. The muscle tubes are $7\frac{1}{4}$ inches long and $1\frac{1}{4}$ inches inside diameter, and their capacity is 150 c c. A graduation is marked at the 100 c c level. In use they stand vertical and the upper end is open, Fig 2. To the lower rounded end is attached a one-fourth inch glass tube which serves as a drain tube. Near the lower end is attached a side tube which serves as a filling tube.

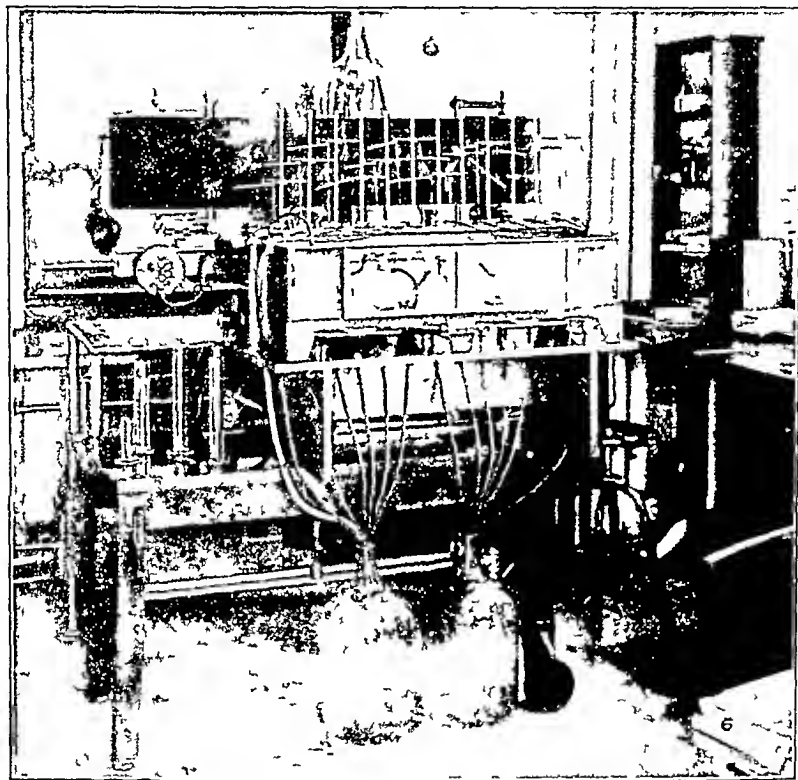


Fig 1—Twelve tube oxytocic apparatus making twelve records on kymograph

The arrangement of the apparatus is quite similar to that described in detail by Dale and Laidlaw in 1912.³ It differs only in some details from the apparatus described more recently by Pittinger and his associates,⁴ Roth,⁵ Hamilton and Rowe,² Eckler,⁶ Burn and Dale,⁷ Smith and McClosky,⁸ Nelson,⁹ and Swanson.¹⁰

The vertical muscle tube is filled to the 100 c c level with Locke's solution (Fig 2). Air is bubbled through the solution constantly by means of a glass air tube which enters the muscle tube from above and extends downward so that the air aperture is nearly at the bottom of the Locke's solution. An L shaped projection from the air tube furnishes a convenient anchor for the lower end of a strip of uterine muscle which is tied to it by a loop of

thread The upper and movable end of the muscle is attached by a thread to the short arm of an Eberbach muscle lever This lever multiplies the movement of the muscle about three times It is counterweighted by attaching small weights to the longer lever arm

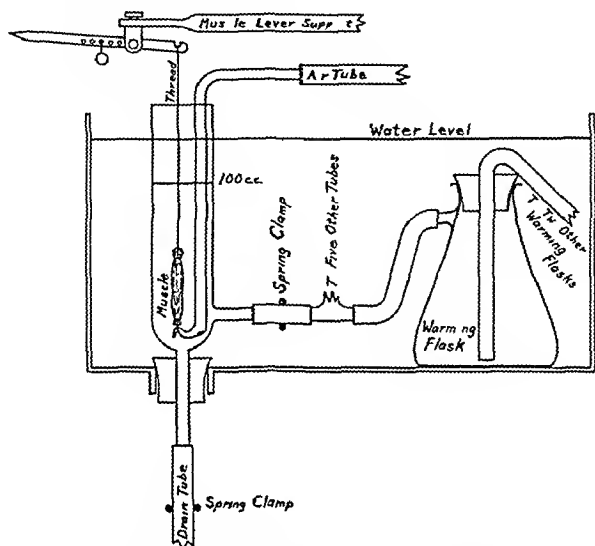


Fig 2—Muscle tube supported by rubber stopper in tubulature of water tank. Uterine muscle anchored to air tube and attached to muscle lever by a thread Warming flask to supply Locke's solution at 38° C for filling muscle tube

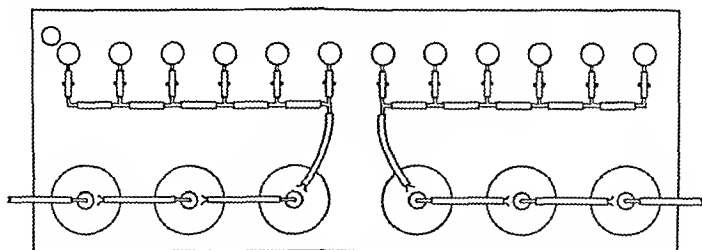


Fig 3—Diagram of 12 muscle tubes in two series each of which is supplied with Locke's solution from 3 warming flasks

The Locke's solution used in the muscle tube is preheated by running it through a series of three warming flasks immersed in the water bath The arrangement of the warming flasks and muscle tubes is shown in Figs 2 and

3 The muscle tubes are emptied by operating the spring clamp or pinchcock on the drain tube and can be refilled with fresh Locke's solution by operating the clamp on the filling tube. This latter procedure requires immersion of the hand in the water-bath which serves the useful purpose of agitating the water to keep it of uniform temperature.

The Locke's solution is supplied from an elevated 20 liter bottle. The air for oxygenating the Locke's solution in the muscle tubes is obtained from the low pressure compressed air line. A header on the air line is equipped with twelve needle valves so that the flow of air through each muscle tube can be regulated independently of the others. The air is bubbled through wash bottles containing a 2 per cent solution of sodium bicarbonate as recommended by Smith and McClosky.⁸

Our thanks are extended to Mr. L. T. Clark, Junior Director of the Medical Research Laboratories, for suggesting the need of a multiple test apparatus and for his interest and help in the design and subsequent use.

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A RAPID QUANTITATIVE METHOD FOR THE DETERMINATION OF ACETONE AND DIACETIC ACID IN URINE*

BY JEANETTE ALLEN BEHRE, PH D., CINCINNATI, OHIO

THE method described in this paper for the quantitative determination of urinary acetone and diacetic acid was designed for use in laboratories where a rapid and standardized procedure is necessary. It is at present in use in this laboratory for the analysis of samples which give doubtful or positive results by the qualitative test recently described in this Journal.¹

Like the qualitative test, this method is based upon the reaction which takes place between acetone and salicylic aldehyde in alkaline solution. The product which is formed has an intensity of color proportional to the concentration of acetone in the solution. The method is essentially the same as that described by Behre and Benedict² for the determination of all the acetone bodies in both blood and urine. The procedure, however, has been simplified as much as possible for the present purpose.

A distillation is required, as no satisfactory method has been found for carrying out the reaction in the urine directly. For the sake of accuracy the volumetric measurements and the distillation should be performed with care, but no elaborate technique is involved. The entire determination can be completed in ten or twelve minutes.

The relative amounts of the reagents used are slightly different from those called for in the original method. A definite amount of urine (10 cc., unless this amount is not available) is distilled to an equal volume. Duplicate determinations may be made on the distillate. Diacetic acid is converted to acetone during the distillation, and determined as such with the preformed acetone, in the distillate.

A set of permanent color standards has been devised, in order to obviate the necessity of keeping standard acetone solutions on hand. These standards involve the use of a side to side, test tube comparison, and for this it is of great importance that the tubes which are used have a diameter accurately measured to correspond with the diameter of the standard tubes. Such a reading is necessarily inaccurate compared with a reading made in a colorimeter, but if carefully standardized tubes are used an accuracy is obtained which is quite sufficient for clinical work. Comparison in a colorimeter is impractical when the permanent standards are used on account of the rapid increase of color which takes place in the final process, as the solution cools. The side to side comparison is made before any cooling has taken place.

*From The Biochemical Laboratory of the Union Central Life Insurance Company Cincinnati.

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The present paper does not include a description of the determination of β -hydroxybutyric acid, but the oxidation of this acid may be carried out as described in the earlier paper² and the resulting acetone determined by the present method

THE METHOD

1 Apparatus —

Water-cooled condenser (250 mm from shoulder to shoulder, straight tube)

Distilling flask (125 or 200 c c capacity)

Glass delivery tube (see Fig 1) to fit with cork stopper onto end of condenser, and drawn out at the other end to reach into receiving tube Curved glass "adapters" may be used for this purpose

Receiving tube, graduated to 10 c c, and of a size to allow the delivery tube to reach to the bottom

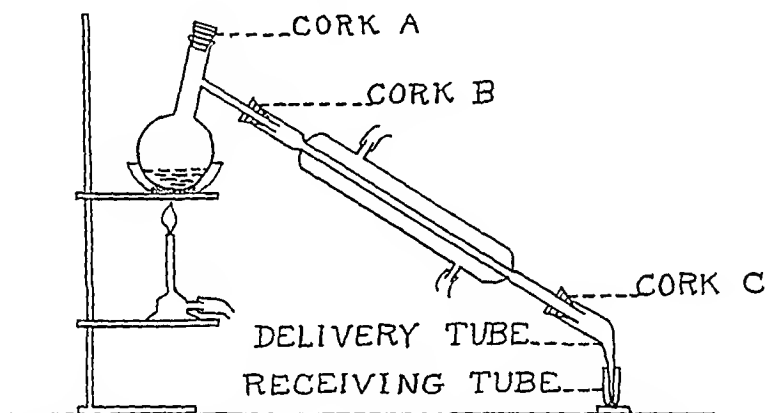


Fig 1 —Distillation apparatus for the quantitative determination of acetone and diacetic acid

Tubes for the determination, of exactly the same diameter as that of the standard tubes (The standardized tubes used for the sulphosalicylic acid albumin determination, obtained from the Fales Chemical Company, are good for this purpose)

Sand-bath and Water-bath

Set of standards Directions for making these are given below

2 Reagents —

Sulphuric acid, concentrated acid added to an equal volume of distilled water

Sodium hydroxide, 32 per cent solution

Salicylic aldehyde, from *Merck* and *Amer* labelled "Acid Salicylicous,

3 *Distillation for the Determination of Acetone and Diacetic Acid*—The apparatus for distillation is first made ready the water connection turned on and the glass tip of the delivery tube on the end of the condenser introduced into the receiving tube which contains a few drops of distilled water, so that the tip of the delivery tube dips below the surface of a minimum amount of water (see Fig 1)

Ten c c of the urine to be tested are measured with a pipette into the distilling flask, and about ten drops of the sulphuric acid and 25 to 30 c c of distilled water are added. The flask is *tightly* stoppered with a good cork stopper (cork A, Fig 1). The stopper and the neck of the flask should be thoroughly dry, and the stopper should fit perfectly so that none of the volatile acetone can escape.

The flask is then connected with the water cooled condenser by means of a cork stopper fitted onto the arm of the flask (Fig 1, Cork B). This connection should also be very tight. It is very important that all of the corks are dry and tightly fitting. Several corks of the right sizes (A and B) may be kept in reserve, with holes bored in B, so that dry ones may always be used. Rubber connections must never be used in places where the acetone may come in contact with them, as rubber reacts to give undue color in the determination.

The sand bath is arranged under the flask, the burner lighted under it and distillation carried on. The boiling should not be so violent that the urine goes over without distillation but a good flame should be used to prevent sucking back. If this should occur the delivery tube at the end of the condenser may be loosened a little to let in air at this point.

When the volume of the distillate has almost reached the 10 c c mark the delivery tube is disconnected from the condenser and blown out into the receiving tube. It may also be washed down with a few drops of water and drained into the receiving tube. The volume is made to just 10 c c. The burner is turned off *after* the delivery tube has been disconnected. The receiving tube is then inverted several times to mix thoroughly.

If 10 c c of urine are not available for the distillation a smaller volume may be used and distilled to an equal volume (marked on the receiving tube). If as little as 5 c c are used and distilled to 5 c c the recovery of acetone in the distillate is almost but not quite, complete.

4 *Determination of Acetone in the Distillate*—Two c c of the distillate are transferred to one of the regular acetone tubes with a 2 c c pipette. 2 c c of 32 per cent sodium hydroxide are added from a burette and 2 drops of salicylic aldehyde (Eimer and Amend) from a dropping bottle. Not more than 2 drops are to be added if more are dropped in by mistake this portion of the distillate should be discarded and 2 c c more taken. The tube is shaken from side to side and put into a boiling water bath for three minutes. Shortly after it has been put in, it should be shaken again from side to side so that all of the salicylic aldehyde is dissolved and the contents of the tube well mixed. The tube may be left in the bath longer than three minutes, but as soon as it

is removed it must be compared with the standards. The hot water on the outside of the tube is wiped off and the comparison made *immediately*. The solution darkens rapidly when allowed to cool, and the reading will be too high if made even two minutes after the tube has been taken from the bath. If there is an unavoidable delay in making the reading, the tube may be returned to the water-bath and left for several minutes, after which the increase in color has disappeared, and the reading may again be made as soon as the tube is removed from the bath. The tube should not be left in the bath for more than ten minutes at most, however.

The reading consists of a side-to-side comparison with the standard tubes, by transmitted light. The percentage of acetone, from diacetic acid and acetone, in the original urine is read directly from the label on the standard tube which most nearly matches the color given by the distillate.

If the acetone content is greater than that represented by the highest standard (0.01 per cent) the distillate may be diluted and the colorimetric determination repeated.

The presence of formaldehyde in the urine interferes to a certain extent with the full development of color in this reaction. If a preservative has been used, such as urotropin, which develops formaldehyde, the reaction of the urine should be made almost neutral, or very slightly acid, before distillation, the addition of sulphuric acid being omitted.

5 Standards for the Color Comparison—These standards were made to match the color produced in acetone solutions treated by the colorimetric method described above, using Eimer and Amend's salicylic aldehyde. The acetone content of the solutions used for comparison was determined by iodometric titration.

We have kept these standards for almost a year in this laboratory, at the present time, without finding any change in their color. It is best to keep them in sealed tubes. Tubes of the standard size may be drawn out at the end, the standard solution introduced and the tubes sealed off.

The formulas for the standards to match various percentages of acetone follow. Only ep chemicals (crystals) should be used.

Blank (color given by the reagents without acetone)—Four hundredths gm potassium bichromate + 0.06 gm potassium chromate, made to 100 cc with distilled water.

0.001 per cent (0.001 gm acetone per 100 cc urine)—One-tenth gm potassium bichromate + 0.6 gm cobalt chloride, to 100 cc with distilled water.

0.002 per cent (0.002 gm acetone per 100 cc urine)—Thirteen hundredths gm potassium bichromate + 1.32 gm cobalt chloride, to 100 cc with distilled water.

0.003 per cent (0.003 gm acetone per 100 cc urine)—Two tenths gm potassium bichromate + 2.0 gm cobalt chloride, to 100 cc with distilled water.

0.004 per cent (0.004 gm acetone per 100 cc urine)—One and three tenths gm potassium bichromate + 1.3 gm cobalt chloride, to 100 cc with distilled water.

0.005 per cent (0.005 gm acetone per 100 cc urine)—One and seven-tenths gm potassium bichromate + 1.7 gm cobalt chloride, to 100 cc with distilled water.

0.0075 per cent (0.0075 gm acetone per 100 cc urine)—Three gm potassium bichromate + 3.0 gm cobalt chloride, to 100 cc with distilled water.

0.01 per cent (0.01 gm acetone per 100 cc urine)—Three and eight tenths gm potassium bichromate + 3.8 gm cobalt chloride, to 100 cc with distilled water.

SUMMARY

A method is described for the quantitative determination of urinary acetone and diacetic acid, suitable for use in clinical laboratories

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A COLORIMETRIC METHOD FOR THE DETERMINATION OF BILE ACIDS IN URINE*

By ICHIRO KATAYAMA MD NEW YORK CITY

THE lack of a satisfactory method for bile acids in urine has been an impediment to a comprehensive study of bile acid metabolism in pathologic states in man. The elaborate technique utilized by Schmidt and his coworkers for the bile acids of bile and urine of experimental animals are not readily applicable in clinical laboratories. The principles of the method proposed by Szilard¹ for the estimation of bile acids in blood have been adapted to analysis of urine. In order to separate the bile acids from interfering substances, advantage has been taken of the observation made by Tengstrom, Schmidt and Merrill² that bile acids are precipitated from solution by saturating the solution with magnesium sulphate.

METHOD

An alcoholic solution of the bile acids of the urine prepared by adding 50 cc of absolute alcohol to 10 cc of urine, is heated in a boiling water bath to effect solution of the bile acids and to coagulate the proteins. This is filtered and the filtrate is evaporated at about 70° C to dryness in vacuo. The residue is dissolved in about 15 cc distilled water and the bile acids are precipitated from their aqueous solution by the addition of solid magnesium sulphate to saturation. After standing in the ice box overnight, the residue is filtered on the best quality filter paper and then washed with small quantities of a cold saturated solution of magnesium sulphate. After the filter paper has been dried completely, the bile acids are extracted overnight with 80 cc of absolute alcohol. This filtrate is evaporated in vacuo to 10 cc and 200 cc of absolute ether are added to the residue. This mixture is allowed to stand overnight. The bile acids are precipitated by this alcohol ether mixture. The supernatant fluid is removed by siphon without disturbing the precipitate. When entirely dry, the precipitate is dissolved in 5 cc of water transferred to a 15 cc centrifuge tube and 0.3 cc of 1 per cent ferric chloride solution is added and the solution thoroughly mixed. On standing in the

*From the Department of the Laboratories New York Post Graduate Medical School and Hospital.

Received for publication February 3 1928

incubator at 38° C overnight the bile acids combine with the ferric chloride and are precipitated as ferric salts. The mixture is centrifuged and the supernatant fluid removed by siphonage. After the precipitate has been washed twice with water, it is dissolved in 5 c c of absolute alcohol and quantitatively transferred to a test tube, using 3 c c absolute alcohol. To this test tube 5 c c of chloroform and 5 c c of sulphosalicylic acid reagent are added. After thoroughly mixing, the stoppered tube is permitted to stand in a bath at 40° to 50° C with occasional shaking for two to three hours, i e, until the purple color in the upper layer has reached its maximum intensity.

A stock solution of 0.1 per cent glycocholic acid may be kept for about one week. From this stock solution a series of four standards for comparison are prepared by pipetting into 15 c c conical centrifuge tubes 2.0 c c, 1.5 c c, 1.0 c c, and 0.5 c c, respectively, of the stock solution. The contents of each tube is diluted to 5 c c and to this solution 0.3 c c of the ferric chloride reagent are added. The stoppered tubes containing the standard solutions are incubated at about 40° C overnight. From this point the procedure for the development of the color in the standards is identical with that for the unknown. There are available four standards for comparison in the colorimeter containing 2.0 mg, 1.5 mg, 1.0 mg, and 0.5 mg of glycocholic acid.

To obtain the most satisfactory results it has been found advisable to keep the colorimetric reading within a narrow range, about 15 mm. This is accomplished by a comparison of the unknown with the standard most closely approximating it in color intensity. A sample from the upper colored layer of both standard and unknown is pipetted into the colorimeter cups for comparison.

Calculation $S = \text{mg of bile acid in the standard used for comparison}$

$$\frac{15}{R} \times S \times \frac{\text{vol of mine}}{\text{used mine vol}} = \text{mg of bile acids as glycocholic acid per volume of urine}$$

Reagents—Ferric chloride. 1 per cent of ferric chloride containing 0.05 per cent of concentrated hydrochloric acid.

Sulphosalicylic acid. 8 grams of sulphosalicylic acid in 1,000 c c of 0.01 N hydrochloric acid.

A satisfactory recovery of the bile acids in aqueous solution and of bile acids added to urine is indicated by the data in Tables I and II.

TABLE I
RECOVERY OF TAUROCHOLIC AND GLYCOCHOLIC ACID IN AQUEOUS SOLUTION

BILE ACID	THEORETIC MG PER 100 C C	FOUND	RECOVERY PER CENT
Glycocholic	15.0	14.3	95
Glycocholic	10.0	9.5	95
Taurocholic	15.0	15.1	100
Taurocholic	10.0	9.9	99
		Average recovery	97.5

TABLE II
RECOVERY OF TAUROCHOLIC AND GLYCOCHOLIC ACIDS FROM URINE

PRESENT IN URINE	BILE ACID ADDED MG PER 100 CC	FOLD	RECOVERY PER CENT
50	15 glycocholic	197	98
00	10 glycocholic	93	93
50	15 taurocholic	201	100
00	10 taurocholic	98	98
Average recovery			97

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A NOTE ON THE TECHNIC FOR STAINING THE NUCLEAR MATERIAL IN BLASTOMYCETES*

By EVERETT S SANDERSON PH D CHARLOTTESVILLE, VIRGINIA

WHILE engaged last year in the making and assembling of photographs from a case of systemic blastomycosis admitted to the University of



Fig 1



Fig 2



Fig 3



Fig 4

Blastomyces in the yeast stage stained with hematoxylin and showing the arrangement of nuclear material. From slant agar cultures three to five days old. Oil immersion $\times 1500$. Figs 1, 3 and 4—Various stages in the budding process. Fig 2—An unstained organism for comparison. Figs 5 and 6—Transformation from yeast stage to mycelian form, unstained.

From the Department of Pathology and Bacteriology, University of Virginia.
 Received for publication February 5, 1925.

Virginia Hospital, which were used as a part of the exhibit at the annual meeting of the American Medical Association,* it was felt that photographs of hematoxylin-stained organisms, to bring out the nuclear material during the process of budding, would be of added interest. It was found, however, that when smears of the blastomyces were dried on slides and stained in the usual way the thick outer capsule caused shrinking and distortion to such a degree that they were entirely unsatisfactory for photographic purposes. Several



FIG 5

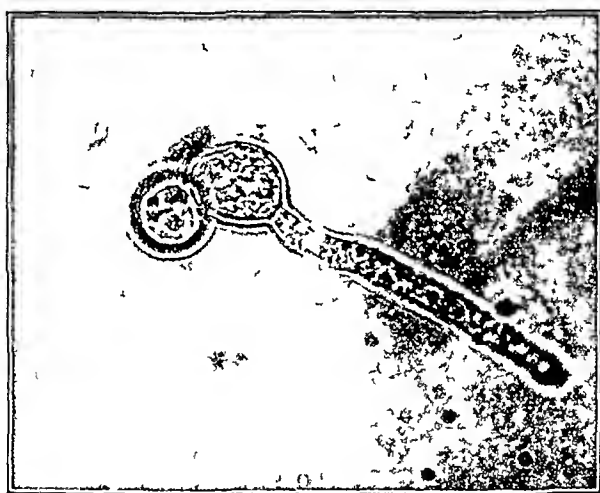


FIG 6

methods were tried to alleviate this difficulty, and the one here described gave very satisfactory results.

The organisms in the yeast, or budding stage, were washed off agar slants with water and then fixed by boiling in a small centrifuge tube for from thirty to fifty seconds. The emulsion was centrifugated, the supernatant fluid discarded, and the organisms resuspended in from one to two cubic centimeters of hematoxylin and stained for from two hours to overnight, depending upon the strength of the stain. At the end of this period the excess dye was removed by alternate centrifugation and washing in tap water, and

*In collaboration with Dr. D. C. Smith of the Department of Dermatology and Syphilology, University of Virginia.

to the last washing a trace of ammonia added to bring out the desired color. If permanent slides are desired, the organisms can be mounted in a glycerin jelly. Figs 1 to 5 show the results to be had by this method.

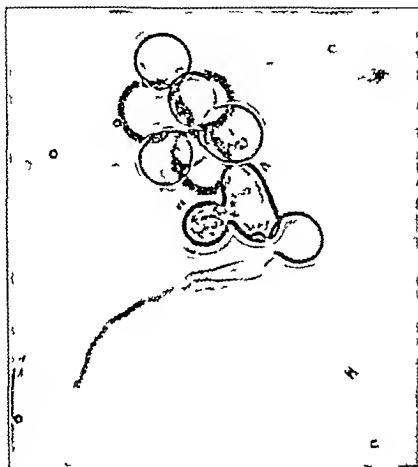


Fig. 1

Two other photographs of unstained organisms are included to show the transformation from yeast type to mycelium form, the former being the one usually observed in tissues, while the latter is responsible for the 'cottony' growth seen in cultures.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

IMMUNITY Experimental Immunization With Bacteria Detoxicated by Gold Chloride,
Osman, M. B. Jour Immunol, 1927, *vi*, 3, 249

Detoxication was carried out as described by Sanfilippo, by suspending growths of the various organisms in a 1 per cent solution of gold chloride. Organisms such as *B. typhosus*, grown on solid media, were emulsified in a known quantity of sterile distilled water to which an equal volume of 2 per cent gold chloride was added. In the case of streptococci and pneumococci which were grown in a veal extract phosphate bouillon, the organisms were deposited by centrifuging, the supernatant bouillon was completely removed (to avoid any formation of a precipitate on the addition of the gold solution) and the growth was then emulsified in distilled water to which 2 per cent gold chloride was added, as before. Immediately after the addition of the chemical, the organisms tended to agglutinate but the clumps could be broken up by shaking, thus allowing the gold salt to act uniformly. The treatment was allowed to proceed for one hour. The emulsions were then centrifuged and the deposit was washed with sterile distilled water. Clumps were again broken up to ensure thorough washing, which was repeated three times. After the third washing, the organisms were suspended in the minimum amount of distilled water.

For purposes of dosage in immunizing animals, the number of organisms per cubic centimeter of the suspension was estimated by means of Brown's opacity standards. When large doses were to be injected, dense emulsions were made up and their concentration was computed by preparing a 1:10 dilution whose opacity fell within the range of Brown's standards. The number of organisms in the original suspension could then be estimated and the required dose given in small bulk.

In immunizing animals, all injections were given intravenously.

Gold chloride exerts a marked detoxicating effect on various bacteria.

The power of such detoxicated bacteria to stimulate agglutinin production is slight.

Animals immunized with such detoxicated organisms tolerate large doses of live organisms.

By immunizing with a large dose of detoxicated organisms, followed after a week by a large dose of live organisms, an agglutinating antiserum of high titer can be readily obtained.

FECES A Rapid Method for the Detection of Ova and Cysts of Intestinal Parasites,
Rivas, D. Jour Trop Med, 1928, *vi*, 1, 63

1 Place 1 to 2 gm feces in a medium or large sized test tube and add 5 c.c. of 5 per cent acetic acid for each 5 gm. of feces.

2 Stopper tube and shake until suspension is homogeneous.

3 Allow to stand one minute for coarse particles to settle.

4 Remove supernatant fluid with a pipette or, if necessary, filter through double layer of cheesecloth.

5 Place 5 c.c. of filtrate in centrifuge tube, add an equal volume of ether, stopper and shake horizontally until homogeneous (a few seconds).

6 Centrifuge for one to two minutes.

7 Pour off all but the sediment (which may be very scanty).

8 Mount and examine the sediment.

FECEES EXAMINATION Technic of Examination of Feces for Amebas and Protozoa

Albert H Arch Path and Lab Med, 1927, iii 227

1 Iodine Eosin stain of fresh material A drop of physiologic sodium chloride solution and one of iodine eosin stain are placed near together on a slide but not touching A round applicator stick or a toothpick is smeared with the feces and rolled in the drop of physiologic sodium chloride solution and then in the drop of iodine eosin A single cover slip is placed on both drops half the material under it being stained and the other half unstained The unstained portion should be examined first for living flagellates and active amebas In the stained portion the protozoan cysts stand out as bright spherules against the pink back ground and soon become tinged with the iodine to varying tones of yellow with the nuclei becoming clearly defined as the iodine penetrates If glycogen is present in the cysts it becomes light or dark brown

Iodine eosin stain consists of saturated aqueous solution of eosin in physiologic sodium chloride solution 2 parts 5 per cent solution of potassium iodide in physiologic sodium chloride solution saturated with iodine 1 part physiologic sodium chloride solution 2 parts

The proportion of iodine solution used may be modified to advantage by adding a slight excess of that given in the formula if the nuclei do not appear after a few moments' application of the stain The stain should be made up each day from the stock ingredients

If no bright spherules stand out against the pink background or no other evidence of protozoa is found in two preparations examined, the specimen is reported as negative If bright spherules (cysts) or living protozoa in the vegetative stage are found several smears are made and stained with hematoxylin using the following method

2 Hematoxylin stain of fixed material A smear is made on a slide which has previously been thoroughly cleaned in alcohol ether and flamed If the fecal material is too dry, it should be moistened slightly with physiologic sodium chloride solution and a thin smear made with the applicator stick or the flat side of a toothpick or by using the edge of an other slide or a cover slip It should then be immersed directly in Schaudinn's fixing fluid without allowing the slide to become dry The following steps are used in fixing and staining the preparation

Schaudinn's fluid (2 parts saturated aqueous mercuric chloride in physiologic sodium chloride solution 1 part absolute or 90 per cent alcohol 4 cc glacial acetic acid is added to 96 cc of the mixture on using) (freshly prepared each time) is heated to from 50 to 60 C for 10 minutes

70 per cent alcohol tinged with Gram's iodine	5 minutes
70 per cent alcohol	5 minutes
50 per cent alcohol	5 minutes
Tap water	2 minutes
2 per cent iron alum* aqueous solution	5 to 12 hours
or heated to 30 C	10 minutes to 1 hour
Tap water—rinse	1 minute
5 per cent hematoxylin† aqueous solution	12 to 18 hours
or heated to 30 C	10 minutes to 1 hour
Tap water	1 minute

Differentiate in 1 or 2 per cent iron alum with careful watching under microscope (Do not allow to dry!) (After placing in alum solution about a minute wash in water then make final examination)

Wash in running water	10 minutes
50 per cent alcohol	10 minutes
70 per cent alcohol	5 minutes

*Use only violet crystals of iron alum. Reject yellowish powder
†Use American hematoxylin standardized white crystals only

90 per cent alcohol	5 minutes
100 per cent alcohol	5 minutes
Xylol	5 minutes

The preparation is then mounted in balsam and covered. Examination is made preferably with a binocular microscope using the oil immersion lens.

SPIROCHETA PALLIDA Method for Demonstration in Single Sections, Dieterle, R. R.
Arch Neurol and Psychiat, 1927, *xxviii*, 73

Reagents

Uranium nitrate	5 gm
70 per cent alcohol	500 cc
Gum mastic solution	
Gum mastic	10 gm
Absolute alcohol	100 cc

Allow to stand with frequent shaking for three days or until dissolved. Filter through three-fold filter.

Silver nitrate solution

1 per cent aqueous (protect from light)

Developing solution

Hydroquinone	15 gm
Sodium sulphate	0.25 gm
Merk's Blue Label Formaldehyde (neutral)	10 cc
Acetone	10 cc
Pyridine	10 cc
Water to make	90 cc

Mix, dissolve, and add 10 cc of 10 per cent alcoholic gum mastic solution to make the mixture "milky."

Method (Applicable to frozen, paraffin, or celloidin sections after formalin fixation)

- 1 Place sections in the alcoholic uranium solution at 55° C for one hour.
- 2 Wash for a moment in distilled water.
- 3 Pass through 96 per cent alcohol.
- 4 Handling the sections individually, place them in the gum mastic solution for about thirty seconds. Then immerse for an instant in 96 per cent alcohol.
- 5 Transfer to distilled water.
- 6 Silver nitrate solution one to six hours at 55° C protecting from light (overnight at 37° C).
- 7 Wash for a moment in distilled water.
- 8 Place in developing solution five to fifteen minutes.
- 9 Rinse in distilled water.
- 10 Pass through 96 per cent alcohol and acetone, several changes.
- 11 Xylene and mount in balsam.

Frozen sections should be made after twelve hours' fixation in 10 per cent formaldehyde. After passing celloidin sections through acetone, and paraffin sections through Xylene and alcohol, proceed with Step 1.

Smears from a primary lesion or tissue emulsion are placed in 10 per cent formalin in 95 per cent alcohol (10:90) for one hour, rinsed in water and then started with Step 1.

The spirochetes are golden-yellow to golden brown and are best photographed with a red filter, using a light blue for histologic detail.

PARESIS Types of Therapeutic Response Observed in the Malaria Treatment of General Paralysis Kirby G H and Bunker H A Am Jour Psych 1926 vi 2 205

It is possible to divide into three major groups 93 cases of general paralysis who were given the malaria treatment between June 15 1923 and December 1 1925 and who survived the period of treatment by more than two months (1) Those in whom the treatment has apparently been without any effect whatever 13 in number (2) those in whom the results attributable to treatment have been of a more or less temporary character 15 in number and (3) those in whom the therapeutic influence whatever its degree has persisted essentially unmodified up to the present time 65 in number The last two groups are, of course further divisible according to the degree of temporary or permanent improvement Provisionally included among the 65 permanent results are 18 patients who though they have exhibited no improvement have manifested so far after various periods extending up to three years no evidence of retrogression

The better the therapeutic result obtained the more likely it is to be of a more or less enduring character for the ratio of those who evidenced a subsequent decline to those who have so far preserved the status quo is as 8 2 among the only slightly improved cases as 6 9 among the considerably improved and as 1 36 among the full remissions

Of 41 patients followed for more than a year who received no further antisyphilitic treatment during that period a well marked modification in the strength of the spinal fluid Wassermann took place in 13 or in 31 per cent (in 8 of whom the reaction became completely negative) in 11 or 26 per cent the Wassermann reaction was definitely modified in 17 or in 42 per cent it remained unchanged No very striking correlation between clinical status and spinal fluid findings was present save that six of the eight cases to become Wassermann negative had attained full remissions In the eleven other full remissions in this group the Wassermann reaction in the spinal fluid was unchanged or only slightly modified The Wassermann reaction in the blood remained essentially unchanged in 28 of the 30 patients who were positive prior to treatment (or in 78 per cent) in only six cases did the blood become negative with an alcoholic antigen and in only two with a cholesterolized antigen as well

Patients of the manic type of general paralysis exhibited by far the greatest tendency to a favorable response to treatment Of 16 manic cases in this series 13 achieved a permanent result and 12 of these a full remission Of 48 patients of the simple dementing type 30 achieved a permanent result but 15 of these belong in the stationary and unimproved group and only seven attained a full remission

The total duration of the various physical and mental symptoms referable to general paralysis averaged at one extreme twenty seven months in the cases upon whom treatment was without influence and at the other sixteen months in the patients who achieved full remissions There was little difference in this respect between the temporary group and the permanent group as a whole

In the 7 out of 12 patients who did not respond to treatment a more or less progressive loss of weight took place subsequent to treatment 80 per cent of the temporary cases eventually lost much of the weight which they had previously gained 48 per cent of the 'permanent' cases registered a more or less progressive gain in weight without subsequent loss In the case of temporary gain in weight all three groups of patients made a gain of equal magnitude amounting to about 16 per cent of the pretreatment weight, on the average but the cases uninfluenced by treatment and the temporary cases subsequently lost about 80 per cent of this gain whereas the permanent cases lost only 48 per cent When this posttreatment gain in weight was sustained without subsequent loss the amount of this gain was likewise about 17 per cent of the pretreatment weight on the average

Among the clinical factors which seem to play a part in the results obtained by the malaria treatment the so called clinical type of general paralysis appears to rank first in importance the behavior of the body weight subsequent to treatment second and the duration of the symptoms prior to treatment third

*An Illustrated Guide to the Sht Lamp**

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The real cerebral birth injuries are caused by the rarer intracerebral hemorrhages and necroses, depressed fractures with brain laceration and by encapsulated hematomas All the evidence collected by Ford indicates that the true cerebral birth palsies are represented by congenital hemiplegias, monoplegias, and the asymmetrical and unequal bilateral spastic paralyses Eight case reports and a list of 136 references accompany this section

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First, cord injuries are due to a type of force never imposed except during labor, the second factor has to do with real but rather vaguely defined differences between fetal and adult tissues

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Certain peculiarities of style attract the reader's attention. This handbook is intended for the use of men and women who may find themselves in the position that they have to undertake the care of the sick injured or wounded. . . . see that there is a provision of a W.C.s etc.

In Chapter XI Children's Ailments one notes paragraphs devoted to the treatment of 'growing pains' and under headache a suggestion to find the cause which one might venture to say might at times be a somewhat difficult matter for 'men and women' no matter how earnest their desire to care for the sick.

This book, in the reviewer's opinion contains too little for the nurse and too much for the layman and suggests a type of volume of dubious value namely the sort of text intended for the lay treatment of disease in the home.

Bacteriology, Blood Work, and Animal Parasitology. By E. R. Stitt, M.D. Surgeon General U.S. Navy. Eighth Edition. Cloth 1 plate and 11 other illustrations containing 683 figures. P. Blakiston's Son & Co.

†*A Manual of Chemistry.* By W. Simon, M.D. and Daniel Base, Ph.D. Thirteenth Edition revised by J. C. Kraut Jr. Sc.D. Professor of Pharmacy University of Maryland. Cloth 695 pages 25 illustrations and 6 colored plates. Lea and Febiger Philadelphia.

‡*Every Woman a Nurse, Health and Nursing Notes.* By Edith Newsome, S.R.N. Cloth. 704 pages 31 illustrations. Oxford University Press New York.

ACIDOSIS A Reliable Clinical Method for Estimating Acidosis, Breed, L. M. Jour. Am. Med. Assn., October 30, 1926, LXXXV, 1478

Technic One cubic centimeter of freshly drawn whole blood is added to 15 cc of acetone free methyl alcohol in a 25 cc volumetric flask. The mixture is shaken thoroughly and filtered into a beaker of 100 cc capacity. A few drops of phenolphthalein is added as an indicator to the filtrate which is then evaporated, just to dryness, over a water bath. The residue is redissolved in 10 cc of distilled water, and compared with 10 cc of phenolphthalein solution in the same sized beaker. If the color matches, one may know instantly that acidosis is not present, and likewise, if no color is evident, that acidosis is present. For exact figures, the estimation is as follows:

If the color matches the standard and remains for six hours it is marked $\times \times \times$

If it remains for five hours it is marked $\times \times$

If it remains for four hours it is marked \times

If it remains for three hours it is marked $\times - -$

If it remains for two hours it is marked $\times - - -$

If it remains for one hour it is marked $- - -$

If it has no color at all it is marked $- - -$

VALUES FOR COMPARING CARBONATE CONTENT WITH PERCENTAGE BY VOLUME OF CARBON DIOXIDE

CARBONATE CONTENT	CARBON DIOXIDE PERCENTAGE BY VOLUME
Color for six hours, $\times \times \times$	over 72
Color for five hours, $\times \times$	67 — 71
Color for four hours, \times	63 — 67
Color for three hours, $\times - -$	61 — 63
Color for two hours, $\times - - -$	55 — 60
Color for one hour, $- - -$	44 — 48
No color, $- - -$	less than 37

The distilled water must have been titrated with phenolphthalein and kept in a room free from acid fumes, and the redissolved filtrate must be placed in the same room and compared with the standard every hour for completing the estimation by comparing with the table of values.

The phenolphthalein standard solution is made by dissolving 1 cc of Hynson and Westcott phenolsulphonephthalein in 1,000 cc of distilled water. This will keep indefinitely.

BLOOD GROUPING A Simple Method of Testing for Blood Compatibility, Felsen, J. Arch. Path. and Lab. Med., 1927, LV, 4, 552

Blood is collected in two capillary tubes, one of which contains a few grains of powdered sodium citrate, from the recipient (R) and the prospective donors (D 1-2-3, etc.).

The citrated capillary tube is rotated and inverted 20 times to insure thorough mixing and, after the blood is allowed to gravitate to one end, this end is plugged with soft wax or paraffin. Both the plain and citrated tubes are inserted in a cork bored $\frac{2}{3}$ its length and the cells and plasma and clot and serum separated by centrifuging or allowing to stand twenty minutes. Scratch marks are made with a file just above the wax plug and at the juncture of cells and plasma and the tube broken the tip containing the wax plug being discarded. There are now four tubes for each person: (1) citrated cells, (2) plasma, (3) clot, (4) serum.

Using a fine capillary rod (a separate one for each person), place a small drop of recipient's cells and donor's serum on a clean glass slide, and a similar drop of donor's cells and recipient's serum, and mix. Read for agglutination or hemolysis with the naked eye (fifteen minute time limit).

Time is saved if only those donors theoretically compatible, are tested. Grouping is done with the citrated cells and known group sera.

The serum of compatible donors (from the uncitrated capillary tube) is used for a Kahn test and one drop used for an intracutaneous test on the recipient.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building
Richmond, Va

*The Erythrocyte and the Action of Simple Hemolysins**

THE increasing specialization in all fields of medicine, clinical or experimental and the rapidity with which developments occur make it practically impossible for any one author to deal adequately with current advances in knowledge. To meet this situation the textbook is supplemented by the monograph.

Especially is this true of those branches or divisions of the medical art which are the subject of experimental investigation.

This volume is a monographic contribution in a special field concerned with the action of simple hemolysins presenting not only the researches of the author but also a comprehensive survey of all the related contributions which have been made upon the subject.

The book is divided into two sections. The first is devoted to the morphology, chemistry and structure of erythrocytes in general and comprises in 85 pages a very complete survey of the literature on the question and the arguments for and against the various theories advanced concerning particularly the morphology and structure of erythrocytes.

In the second part of the book (95 pages) Ponder presents in more or less detail his researches upon hemolysis and the mechanism of simple hemolysins which he believes to fall into certain well defined groups:

- 1 Acting in a way similar to Saponin
- 2 Acting in a way similar to bile salts
- 3 Acting in a way similar to acids or alkalis
- 4 Producing osmotic changes like hypotonic saline

The action of each of these members is considered in detail and constructively discussed.

The volume is of great interest to those interested in biologic investigations and well repays its perusal.

The Antisterility Vitamine Fat Soluble E†

THIS monograph is a direct continuation of an earlier series of papers by Dr Evans in collaboration with Dr R. S. Bishop and details the studies of the authors upon a specific vitamine discovered in the laboratories of the University of California and found to be essential for the normality of reproduction in mammals.

From extensive studies upon rats the authors are convinced that there is a substance present in the active fractions of their vitamine fat soluble E which is specific for the cure of a specific type of sterility induced by certain purified diets.

Their experiments are fully described and the evidence adduced is of the utmost interest. The report is excellently prepared but it is regrettable that its large size (13 by 10 inches) renders it somewhat unwieldy to handle comfortably.

The Erythrocyte and the Action of Simple Hemolysins. By Eric Ponder. Lecturer in Physiology, Univ. of Edinburgh. Cloth 19 pages 11 figures. Oliver and Boyd London England.

†The Antisterility Vitamine Fat Soluble E. By E. H. McJ. Evans and G. O. Burr. Paper. XII microphotographic plates 107 tables 2 graphs 3 text figures. Volume VIII. University of California Memoirs. University of California Press Berkeley.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

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Every Woman a Nurse. Health and Nursing Notes. By F. H. Newsome S. R. N. Cloth. 64 pages 31 illustrations. Oxford University Press New York.

*Normal Midwifery for Midwives and Nurses**

IN THIS book is embodied the lectures delivered by the author to nurses while he was Assistant Master at the Rotunda Hospital

In the introductory chapter occur these paragraphs which at once assure the reader of a clear, sane, and eminently practical discussion of the subject

"One thing is absurd, and that is to try and improve matters by increasing the theoretical knowledge of the midwife" (One is strongly tempted to add that this applies with equal force to the nursing curriculum which is almost daily increasing in scope, at least, in America) "A Sister in the medical world is not expected to discuss the pathology of bronchitis, to percuss a chest, or listen for râles or crepitations, neither should a midwife be expected to know anything about the pathology or treatment of abnormal labor. She should be able to recognize that the labor is abnormal but should not be required to make any diagnosis"

This is a student's text deserving of wide circulation and one which could well be read by many nurses who are, unfortunately, no longer students

Potassium and Tartrates†

A COMPREHENSIVE review and summary of the literature concerned with the physiologic effects of potassium and tartrates of especial interest to therapists, the first part of the book (103 pages) being concerned with the effects of potassium, and the second part (51 pages) discussing tartrates

The monograph is a comprehensive summary of the subject discussed

Malarial Psychoses and Neuroses‡

WITH the exception of those whose field of practice has carried them into appropriate localities, it is entirely possible that to many, malaria means "chills and fever"

"It is remarkable," says the author in his Preface, "that although malaria is traceable two thousand five hundred years old, that although it is the oldest disease of which we have any reliable record, that although it is the most widespread disease in the world today, and that although its most characteristic feature, the paroxysm, which through its periodicities gives the varieties of its forms their names, is largely a neurologic phenomenon, there appears to be no comprehensive work dealing with the nervous manifestation of it"

This hiatus he has endeavored to fill in the present volume which is, indeed, a comprehensive and scholarly monograph

After a brief but clear description of the parasite and the disease, there follows an interesting discussion of malaria in history and its effects upon character and race degeneration

A well-written section on pathology follows in which are found most of the illustrations. These are mainly microphotographs and colored drawings, well chosen and excellently reproduced. The clinical pathology of the endocrine and other glands is then taken up after which a varied array of malarial psychoses are separately considered

There are well written chapters on malaria and alcohol, malaria and surgery, malarial nerve conditions, and malaria in its medicolegal relations, some two hundred illustrative case histories being briefly but clearly summarized

The author has evidently had a comprehensive experience and speaks with authority

*Normal Midwifery for Midwives and Nurses By G W Theobald Cloth 258 pages 28 illustrations Oxford University Press New York

†Potassium and Tartrates A Review of the Literature on Their Physiological Effects By R. W Webster M.D Cloth 168 pages The Commonwealth Press Chicago

‡Malarial Psychoses and Neuroses By W K Anderson M.D Recognized Teacher of Clinical Medicine Glasgow University etc Cloth 395 pages 18 illustrations 4 colored plates Oxford University Press New York

The literature of the world has been carefully and intelligently searched and the writings of the ancients have not been neglected thirty four pages at the end of the book list the references

The author is to be congratulated on the completion of a work which will last and the publishers upon its presentation in a worthy and attractive form

*Poverty Nutrition and Growth**

THIS volume Special Report 101 of the British Medical Research Council is the report of a Committee detailed to study the problems of child life. The work was begun in 1919 under the general supervision of the Scottish Committee for Child Life Investigation and summarizes an enormous amount of data secured by almost innumerable labor

Part I details the general plan of the work the populations studied and the methods employed. The general description of the slum districts covered in these investigations would open the eyes of American Labor disgruntled with its lot. Part II embodies a comparison between the children of the poorer classes in town and country. Part III a consideration of factors generally supposed to influence the nutrition and growth of children. Part IV in come and home conditions. Part V diet. Part VI Parental factors. Part VII the children of rural miners and Part VIII the children of agricultural laborers.

An appendix lists the various forms etc. used in the investigations. If only for its value in teaching how the other half lives this report deserves wide circulation.

The data are of great value and interest to sociologic workers and indeed to pediatricians and all interested in human welfare.

The evidence does not substantiate any marked delay in growth as a result of poverty per se but shows that small parents tend to beget small children and that the small size of the town child is an inborn characteristic.

There was no clear indication that nutrition was directly associated with income. A correlation of weight with breast and artificial feeding was manifest up to eight or nine months after that it was absent.

The most significant and constant finding was the correlation between maternal efficiency and the height and weight of the child. Overcrowded dwellings and an inferior type of mother tended to go hand in hand. This pioneer study as is usually the case raises more questions than it answers and points the way to problems for future investigations.

It is impossible in a short review to indicate the volume of data contained in the report. It must be read to be grasped and studied to be appreciated.

A Textbook of Biological Chemistry†

THIS book is intended for the elementary student and not for reference and as such amply fulfills the purpose for which it is intended.

Poverty Nutrition and Growth. Studies of Child Life in Cities and Rural Districts of Scotland. By D. N. Paton M.D. Professor of Physiology University of Glasgow and L. Findlay M.D. Professor of Pediatrics University of Glasgow. Paper 333 pages numerous tables and graphs. H. M. Stationery Office London.

A Textbook of Biological Chemistry. By J. B. Sumner Assistant Professor of Biological Chemistry Cornell University. Cloth 283 pages 4 figures. The Macmillan Co. New York.

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EDITORIALS

Pillars of Salt

AS THE study of scientific medicine progresses one fact assumes pre-eminence. To be of real service to the patient the physician must diagnose the patient's disease before its ravages have laid waste his vital organs. Diseases must be diagnosed in their incipency, long before there are organic signs and symptoms, and often before there are marked functional disturbances. Our only chance to do this is by a better understanding of the body chemistry which includes the chemistry and functions of the endocrine glands. As the food makes the blood and the blood feeds the cells, it is necessary to know something of the chemistry of food and digestion. What constitutes nutrition, and what is the difference between nutrition and stimulation? How often is a condition of so called "health" really the mask of stimulation that later destroys the vital organs?

When we try to classify our foods it is difficult to know where to begin. Organic or colloidal foods, inorganic or crystalloidal foods, raw foods which may come under the heading of hydrophile colloids and cooked foods which

may come under the heading of hydrophobe colloids and inert foods which do no more than serve as ballast. Then there are substances which in small doses stimulate and poison insidiously and in larger doses poison and cause deterioration of vital organs. Inasmuch as the physiologists believe that most foods should be of a colloidal or organic nature and that the chemistry of digestion is a problem of colloid chemistry, our attention is drawn to a colloidal substance that is now being consumed in great quantities. Its popularity has even been enhanced of late because we can now obtain it in the "iodized" state. I refer to sodium chloride inorganic.

Long ago it was observed that in certain states of organic deterioration salt seemed to aggravate the condition. Now we know that it interferes with the elimination of certain waste products of metabolism whereas in earlier days it was noted that the nephritic patient grew more edematous, the "Salt Rheum" increased in ichthemia or eczematous states. Haig showed that it interfered with the elimination of uric acid products. Later it was shown that in animals such as dogs and in birds such as chickens where a good deal of the nitrogen is eliminated as uric acid the result of feeding salt, even in very small quantities was death. Antopsy showed the liver and kidneys studded with uric acid concretions.

Yet, what is the most common argument for salt eating? That animals like it, need it even travel miles to so called 'salt licks' to get it. How do we know that these animals are not merely starved and lick salt as a poor substitute for browsing leaves and twigs? Even when we give the horse plenty of salt he still chews the bark off trees, gnaws his manger boards and rasps the telephone poles. Does the fact that animals like salt mean that they need it any more than that we human beings need strong coffee because we like it? Introduce a horse to sugar and give him his choice of a sugar mash or a salt mash. He will gorge himself with the sugar mash and ignore the salt mixture. Does that mean that horses need sugar? At best the argument is most flimsy and would not have a leg to stand on were it not for the fact that salt is a stimulant. It makes us feel good by elevating the blood pressure a little and stimulating the adrenal glands with the result that the cheeks and ears glow. It also brings a happier mental state through a feeling of warmth and good health. We say that salt is necessary for life. But is it? Benjamin Rush found the American Indians as healthy as Stefansson found the Eskimoes or as Bartholomew found the Chinese of the Interior none of whom ever ate salt.

In the days of our forefathers salt solution was used as an embalming fluid. The ancient Egyptians used oils, spices and salt in their mummy wrappings. Today we mummify the living with salad dressings made of mineral oils, spices and salt. You can see any number of these mummies walking the streets. The dried and shriveled bodies and white hair bespeak the hardened livers and sclerotic kidneys. I often wonder why it is necessary to embalm such bodies after they are dead. They are already 'pulled to the gills'.

It is possible for a person to eliminate salt quite rapidly through channels such as the skin and kidneys. As long as the body is strong, the resist

ance good and the glands of internal secretion adequate, not much salt is retained. But when the channels of elimination are inadequate salt retention, with its attending harmful consequences, results and the liver or kidneys or skin, or all three, may show at first functional derangement followed by organic destruction. Albumin, casts, red blood cells and leucocytes in the urine may be signs of so-called Bright's disease. If they are, may not such extensive kidney destruction be the third stage of salt poisoning? The second stage may be the transient albuminuria after moderate exercise or an unusually heavy meal, the first stage the excess of NaCl in the urine, which causes no signs or symptoms and which occurs when the patient feels good and thinks he is in a healthy state. In the third stage the kidneys are so impaired that salt elimination is greatly interfered with. This is a very poor time to think that by restricting salt in the diet we can accomplish very much for the patient. Somewhere along the line there was a point where the salt began to be dangerous. The railroads have found that the best way to eliminate dangerous crossings is to eliminate crossings. Have the people cross the tracks overhead or through subways. In other words, "stay off the tracks." The eating of inorganic salt is a bad habit. Why not let the plants organize NaCl into a colloid form in their leaves and fruits and roots and stems, and eat it that way? The urine and sweat never show an excess when it is consumed in this form.

The great Mackenzie wrote, "The first appearance of disease in the human body is invariably insidious, with little disturbance of the economy and no visible signs of its presence. By and by the patient becomes conscious that all is not well with him, there is a loss of that feeling of well-being which accompanies the healthy state. Disagreeable sensations arise, at first vague, but later becoming more definite and these may become so urgent that he seeks advice. Still no evident sign of disease may be perceived on the most careful examination. By and by the disease, being situated in some organ or tissue, changes the constitution of that part, so that its presence is now recognized by a physical sign, when the clinical methods usually employed reveal its character."

It is only by careful chemical examination of secretions such as mucus, tears, gastric juice, urine, joint fluids, spinal fluid, and blood that we can arrive at early diagnoses of salt poisoning. Our figures for the normal are all too high, since most of the so called normal cases were early cases of salt retention. Again to quote Mackenzie "There are evidences which would surely indicate the nature of the disease in its earliest stages, were we capable of detecting them." If we are ever to be able to detect these evidences we must look for them as chemical pathologists.

—H B (P G W)

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